CIBA FOUNDATION
COLLOQUIA ON AGEING

Vol. 3. Methodology of the Study of Ageing

A leaflet giving details of available earlier volumes in this series, and also of the Ciba Foundation General Symposia, and Colloquia on Endocrinology, is available from the Publishers.

CIBA FOUNDATION COLLOQUIA ON AGEING

VOLUME 3

Methodology of the Study of Ageing

Editors for the Ciba Foundation

G. E. W. WOLSTENHOLME, *O.B.E.,* M.A., M.B., B.Ch.

and

CECILIA M. O'CONNOR, B.Sc.

With 47 Illustrations

LITTLE, BROWN AND COMPANY

BOSTON

Published in London by
J. & A. Churchill Ltd.
104 *Gloucester Place, W.*1

First published 1957

Printed in Great Britain

PREFACE

Under its eminent Trustees, the Ciba Foundation is engaged in a number of activities, all with the purpose of improving co-operation in medical and chemical research between workers in different countries and different disciplines. At its house in London the Foundation provides accommodation for scientists, organizes conferences, conducts a medical postgraduate exchange scheme between Great Britain and France, arranges a variety of informal discussions, awards two annual lectureships, is building up a library service in special fields including a section on gerontology, and assists international congresses and scientific institutions and societies.

In 1954 the Trustees of the Ciba Foundation decided upon special measures for the encouragement of laboratory and clinical investigations relative to the problems of ageing. Three conferences have been organized with this particular aim in view. Two earlier ones, already published, were on "General Aspects of Ageing" and "Ageing in Transient Tissues". This volume contains the proceedings of the third colloquium in this series—the Foundation arbitrarily uses the word "colloquium" for one in a series of conferences in the same field, and "symposium" for single conferences on isolated subjects. On this third occasion, knowing that many long-term schemes for the observation of changes with age in man and whole animals were under way or about to begin in various centres, it was thought useful to examine the methodology of such investigations.

In the organization of the colloquium, the Director received much kindly advice and assistance from Professor Tunbridge and from Professor Best, who also acted as its Chairman.

The reader must judge for himself the adequacy in scope and technique with which these problems are being tackled. It is hoped that his interest will be stimulated, his curiosity aroused and his own potentialities for research challenged by these comparatively informal, thoughtful discussions.

CONTENTS

List of those participating in or attending the Colloquium on Ageing: "Methodology of the Study of Ageing", 25th–26th July, 1956

J. Baló	Inst. of Pathological Anatomy and Experimental Cancer Research, University of Budapest
I. Banga	Inst. of Pathological Anatomy and Experimental Cancer Research, University of Budapest
C. H. Best	Dept. of Physiology, University of Toronto
F. Bourlière	Laboratoire de Physiologie, Faculté de Médicine, Paris
G. H. Bourne	Histology Dept., London Hospital Medical College, London
E. Braun-Menéndez	Instituto de Biologia y Medicina Exper., Buenos Aires
A. Comfort	Dept. of Zoology, University College, London
G. W. Corner	Rockefeller Inst. for Medical Research, New York
J. F. Danielli	Dept. of Zoology, King's College, University of London
K. J. Franklin	Dept. of Physiology, Medical College of St. Bartholomew's Hospital, London
Constance L. Friedman	Dept. of Anatomy, University of British Columbia, Vancouver
S. M. Friedman	Dept. of Anatomy, University of British Columbia, Vancouver
T. Gillman	Dept. of Physiology, University of Natal, Durban
F. J. Kallmann	Dept. of Psychiatry, College of Physicians and Surgeons, Columbia University, New York
C. V. Kidd	Office of Research Planning, National Institutes of Health, Bethesda, Maryland
P. L. Krohn	Dept. of Anatomy, University of Birmingham
M. Landowne	Gerontology Branch, Baltimore City Hospitals, Baltimore, Maryland
A. J. Lewis	Inst. of Psychiatry, Maudsley Hospital, London
I. Lorge	Inst. of Psychological Research, Teachers College, Columbia University, New York
O. Mühlbock	Netherlands Cancer Inst., Amsterdam
R. Nicolaysen	Inst. for Ernaeringsforsking, Oslo

O. OLBRICH	Geriatric Unit, General Hospital, Sunderland
A. S. PARKES . . .	National Inst. for Medical Research, Mill Hill, London
R. E. TUNBRIDGE . .	Dept. of Medicine, University of Leeds
F. VERZÁR . . .	Physiological Inst., University of Basle
JEAN VERZÁR-MCDOUGALL	Physiological Inst., University of Basle
A. T. WELFORD . . .	Dept. of Experimental Psychology, University of Cambridge
A. ZILLI	Società Italiana di Gerontologia e Geriatria, Florence

CHAIRMAN'S OPENING REMARKS

CHARLES H. BEST

THERE are many groups, in different countries, studying the subject of gerontology. I note that most of these associations of scientists encounter some difficulty in the definition of terms and in the delineation of their field of interest. Our Committee has been examining essays on the problems of ageing, which have been written in various parts of the world. We have experienced the usual troubles but we have not taken these too seriously; at this stage of the development of the study of ageing it is wise to inspect broad fields, to look for soft spots in the wall of ignorance and not to worry unduly about exact definitions. We all realize that we are studying variations with time in what we think is normal function. Even this simple broad statement will undoubtedly be subjected to careful scrutiny.

THE BIOLOGICAL APPROACH IN THE COMPARATIVE STUDY OF AGEING

A. COMFORT*

Department of Zoology, University College, London

THE study of senescence deals with the processes which make some organisms decline in vigour with increasing age. It embraces a group of deteriorative effects which we isolate because they are deteriorative, in other words because we dislike and wish to avoid them, and we undertake it with reference to the senescence of one organism, man, whose rate of ageing it is our object to control.

Fields of study in medical biology usually define themselves in this way, but as a rule the processes which we originally treat together for our own convenience prove later to have some biological unity apart from their interest to man. We normally assume the possibility of some such unity in any study of this kind; much of the information upon which we form hypotheses, and, very often, all or almost all the controlled experiments by which we test them, come from examination of apparently analogous processes in animals.

The cephalopod eye bears a strong superficial likeness to the human eye, and one might learn something of the biology of sight in man by studying it, but since its anatomy and chemistry are in fact quite different from those of mammalian eyes, the amount that we are informed or misled by it will depend entirely on the intelligence with which we make use of comparative study. The progress of endocrinology was held up for years by attempts to identify menstrual phenomena in animals which did not menstruate.

In discussions of this kind it always falls to biologists to irritate their colleagues by pointing out the diversity of

* Nuffield Research Fellow.

animals in all those respects where it would be more convenient if they were uniform. I want to discuss two subjects connected with the methodology of age studies. One is the kind of information which we can expect to obtain from comparative studies, and the other the special problem of devising means by which to relate age changes in vigour to the life cycle of animals, particularly vertebrates—both in ontogeny and in phylogeny.

From the biological point of view, comparative age studies, like other comparative studies, could be expected to give two kinds of information, according to the way in which we conduct them—evidence of the existence of fundamental, or at least widespread, processes which make for senescence in organisms, and evidence of the relationship of the human life cycle to that of non-human vertebrates, especially other mammals. The second of these has had curiously little appeal to biologists, possibly because it appears superficially less likely than the first to give rise to large-scale discoveries, but I would like to suggest that it is at present the more promising, if not the more important undertaking; it is also quite essential to work of the first kind, since it is required to give us an indication of the type of unity in age processes which we are entitled to take as our working hypothesis.

The special problems of comparative age studies are of two quite distinct kinds. Some are purely observational problems, due to the fact that the lifespans of the animals we wish to examine may be appreciable fractions of our own, that old organisms when they exhibit senescence are inherently variable, that relatively few animals can be accurately aged by inspection, and that the changes we wish to observe may appear only in circumstances remote from the normal wild environment of the organism we are studying. Others depend on the nature of the definitions we use, and the analogy which we draw between age phenomena in different species. These phenomena may be similar, or analogous, or convergent, and we have no initial means of distinguishing between them.

A great many theories of senescence were devised in the past on the assumption that the loss of vigour with age must involve a single "fundamental" or "inherent" cellular, chemical, or mystical process common to all multicellular animals, contrasting with the extremely ill-named "immortality" of protozoa, and possibly generalizable to include inorganic substances, species in phylogeny, and whole human societies as well. The study of ageing has suffered very heavily from superficial and abstract analogies of this kind, most of them, like the analogy to mechanical wear, grossly misleading in their application. In fact, while some of the assumptions of Weismann and his contemporaries about senescence in metazoa may eventually prove correct, few if any can be taken for granted in comparative work on ageing. It now seems likely that there are multicellular animals which are capable of indefinite somatic cell replacement; in unicellular animals such as suctorians, where mother and daughter cells can be identified at fission, the age-status of the products is not identical, and individuals have a definable lifespan which can be altered experimentally (Rudzinska, 1952, 1955). This might be the case in other acellular animals. It is not possible to assume that all vertebrates undergo senescence—one of the most interesting comparative problems is to find out whether they do so or not. It is not possible to assume that the underlying pattern of senescence is the same in all mammals. I draw attention to these assumptions because papers which make them are continually published; many of them are in substance the research problems which comparative studies could properly be directed to settle. It may well be that broad generalizations about the nature of senile change and its relation to growth and nutrition can be based on the behaviour of rotifers, or the effects of vitamins on the lifespan of insects, but it may equally well prove to be otherwise, in which case a great deal of effort could be wasted.

If we list the apparent causes of decline in the vigour of animals with increasing age, the processes included in that definition are evidently multiple. There is a theoretical

justification, besides our original preoccupation with man, for seeing a biological unity in these processes, and for accepting a general definition of this kind, but it is in terms of the place which senescence seems to occupy in evolution, not, in the first place, of cytochemistry.

In a population subject to the high mortality which affects most wild animals, the evolutionary relevance of individuals depends on the relative numbers of offspring they contribute, and the selection pressure toward vigour and homeostasis probably declines very rapidly with increasing age (Medawar, 1955*a*). It has therefore been suggested that the common feature of ageing processes in animals is that they represent a "running out of programme", and an escape from the selection pressures which maintain fitness early in life. Where individuals rarely reach even mid-adult life unless protected, the postponement of an adverse effect is as effective as its removal, and this will apply both to selection pressure towards further somatic-cell renewal and probably to the elimination of late-acting lethal genes (Haldane, 1941). If this is so, we might expect the processes which limit the lives of different animals to be diverse. At the same time, since the cells of animals are in general similar in many important respects, it is quite probable that some deteriorative processes at the cellular level are shared by all animals. It is probable, in particular, that the lifespan of any non-dividing cell is fixed—perhaps by processes similar to those which Hinshelwood (1951) found in non-dividing bacteria. If so, any animal containing essential and unrenewable cells would eventually display a loss of vigour from that cause. But it does not follow that this is in fact the cause of the senescence of mammals, either through loss of neurones or through endocrine deterioration. Their senescence might equally depend on progressive changes in the quality of cells produced in regenerating tissues. It might conceivably depend on mechanochemical and non-cellular changes in tissue materials which have a low turnover rate, analogous to the changes which cause the senescence of some trees. But these are hypotheses, not definitions, and the

definitions which we employ must be of such a kind that they do not beg the questions which comparative studies are designed to answer.

Measurement of senescence

If we accept the idea that animal senescence may be heterogeneous, it follows, I think, that the more general the test we use to measure it the better. If senescence is to be treated as I have assumed, its most convenient and general measure is the increase with age in the probability of death. The advantages and reservations of this measure have already been discussed by Medawar (1955*b*). If senescence is defined otherwise, then other measures will follow from the definition. The actuarial measure is that normally used in man, and it measures the total loss of vigour against all stresses and from all causes.

Alternatively, we could measure the decline of one particular kind of homeostasis, such as the resistance to temperature or to drugs, or one of the characters contributing to vigour such as fertility or power of regeneration. Most of these indices show changes against age in most animals, but they do so in varying proportions and in contrary senses. Accordingly decline in vigour is often better seen as an increase in the variance, the coefficient of variation, or some other index of scatter for some or all of the characters of vigour, rather than as a parallel trend in all of them. This is true, for example, of the human blood pressure (Bürger, 1954), where the individual values diverge throughout life. We might expect a similar increase in the variance if we measured the regeneration-rate of tissue in an animal which displayed a slowing of regeneration with age, but which had the property of regenerating previously injured tissues more rapidly than uninjured tissues.

Increased variability is sometimes the only character of ageing organisms which correlates closely with their rising mortality. This difference between young and old animals is somewhat similar to that between hybrid and inbred animals of identical age. The resemblance may not be coincidental,

since in each case the large variance is an expression of poor homeostasis, and this in turn may represent a partial withdrawal of natural selection.

The variance of single characters has been used as a measure of inbreeding depression (Maclaren and Michie, 1954, 1955) and it might in some instances be convenient to use

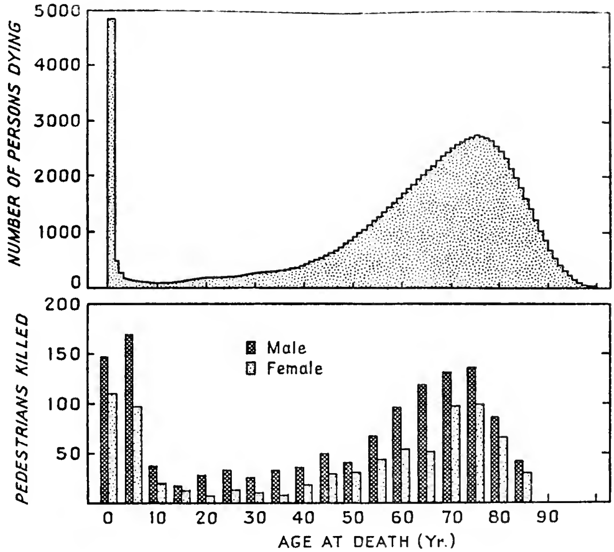


FIG. 1. Distribution by age of deaths from all causes (white U.S. males, 1939–41) and of pedestrian deaths in road accidents (England and Wales, 1950). (Reproduced, with permission, from Comfort, 1956. *Lancet*, ii, 773.)

some such measure of the variance of a number of characters in estimating senescence. As in inbred strains, however, the characters whose variance increases with age are quite irregularly distributed between species and between genetic races of the same species. By measuring the probability of death from the sum total of causes we are using a measure applicable to all organisms, which can be treated mathematically by convenient methods, and which is experimentally consistent—often to a very high degree. In man and in some inbred lines

the pattern of mortality can be substantially affected by diseases which have a characteristic age distribution. But we can see that the crude survival and mortality curves are valid measures of vigour against forces other than these if we compare the distribution of human pedestrian deaths in road accidents (Fig. 1). This depends upon the decline of several characters of vigour; sensory acuity, reaction time, motor co-ordination and recovery after injury. Apart from the fact that we normally exclude infant mortality from the treatment of senescence, we need not therefore follow Pearson (1897) in adopting the five separate Deaths which formed the frontispiece to the first of these Colloquia on Ageing, except in strains of animals or circumstances of culture where one of them grossly predominates.

Animal life tables

If we had a comprehensive account of the relation between growth, development, mortality and chronological age in a sufficient range of representative species, the truth of most of the general theories which have been put forward to explain senescence could probably be tested by inspection. The actuarial studies which we already have, combined with maximum age records, suggest that in invertebrates there is an inverse relationship between degree of cell replacement and liability to senile change. It is now of great importance to obtain accurate data for the relation of age to mortality in the main types of vertebrates, and in as many different species as possible. McCay and co-workers showed (1943) that ageing and general metabolic activity in rats are dissociable, at least prior to maturity, as growth and development are dissociable in the tadpole; it is therefore quite one of the most important problems of age studies to find out, if possible, which components of the developmental "programme" in mammals determine the timing of senescence. Given a sufficient range of comparative information, we might expect to answer this question either directly, from observed correlations, or by a relatively small number of fundamental experiments.

Actuarial senescence almost certainly occurs in all birds and mammals. It is by no means certain that it occurs in all poikilotherms, beyond the accumulation of evident injury with age. The ageing of birds and mammals has been termed "endogenous", and it has been suggested that this type of ageing is in some way connected either with the evolution of homoiothermy or of a fixed adult size. Bidder (1925, 1932) attributed it to the direct action of a growth-inhibitory mechanism evolved during the transition to life on land. From maximum age records it is evident that the lifespan of smaller vertebrates declines fairly steadily from phylum to phylum in ascending phylogenetic order. It is longest in fish, amphibia and reptiles. We have records of 35 years in *Triton* (Smith, 1951) and 40 years in goldfish (Harvey and Hems, 1948). The lifespan of birds is in many cases substantially greater than that of mammals of comparable size and activity. The lifespan of the laboratory mouse is normally less than three years, while that of a chaffinch in captivity may exceed 20 (29—Moltoni, 1947). This suggests that in phylogeny other causes than the increase in metabolic rates have operated to shorten the maximum lifespan—the metabolism of small birds measured by their oxygen consumption is higher than that of rodents, and apparently it does not decline with age like that of many mammals (Benedict and Talbot, 1921). The growth of birds, as Bacon (1645) pointed out, ceases relatively earlier than that of mammals, and much more definitively; the epiphyses of rats never join, and they may continue in growth, or be made to grow in response to somatotrophin, at any age, while no further growth occurs in birds after the attainment of adult size.

There are also important discrepancies between the maximum ages reported in closely related mammals. The most extensive figures are for rodents: thus *Mus bactrianus* has been reported to live and remain fertile longer than any strain of *Mus musculus* (Green, 1932), while species of *Peromyscus* and *Perognathus* live almost twice as long [*Peromyscus maniculatus gambelli* 5 years 8 months (Sumner, 1922);

Peromyscus maniculatus gracilis 5 years 10 months (Dice, 1933); *Perognathus longimembris* $> 7\frac{1}{2}$ years (Orr, 1939)]. These differences are not closely related to size—*Micromys minutus* reaches nearly 4 years in similar circumstances (Pitt, 1955). Leslie and Ranson (1940) and Leslie and co-workers (1955) found a difference in specific longevity between colonies of *Microtus arvensis* and *Microtus orcadensis*, though this might reflect the results of domestication and better culture. Such differences are clearly of great importance to our understanding of age processes, but so far none of the attempts to correlate them with quantities such as the duration of pregnancy or the relative length of pre-adult development is satisfactory—chiefly because the figures assumed for the specific ages of the different species are arbitrarily drawn from maximum age records, some of them quite inaccurate. Most of the valid correlations which can be made out have been reviewed by Bourlière (1946).

In view of questions like these, one of the most important requisites for the understanding of mammalian age processes and the factors which time them is a full range of vertebrate vital statistics, based on animals living under conditions of captivity sufficiently good for a fair proportion of them to reach old age. These figures are almost wholly lacking. So far as can be ascertained, no life table has been published for a captive population of any fish, reptile or amphibian. One incomplete life table exists for domestic poultry, and it is based on an assumed equation to cover losses from culling (Gardner and Hurst, 1933); there is no other table for birds in captivity. Apart from these, we have satisfactory actuarial data only for man, laboratory rats and mice, and a few other small rodents, with partial figures for culled populations of agriculturally important animals [e.g. Merino ewes (Kelley, 1939)]. There are thus no data for any vertebrates other than mammals; the figures which might throw light on the evolution of mammalian senescence, those for poikilotherms, birds and marsupials, have never been sought.

One consequence of this lack of information is that we have

no experimental mammal intermediate in size between man and the small rodents whose rate of ageing is actuarially known. There are no published actuarial data for rabbits: their modal specific age for all strains is probably about 8 years, but large hybrids may reach ages as great as 15 years (Comfort, 1956). Data for guinea pigs have been collected and briefly reported (Rogers, 1950) but we have no comparison of strains. Accordingly, many physiological and other differences described in the literature between young and "old" animals are in fact differences between infant and young adult animals, and even where this is not so it is impossible to establish correlations between such changes and the rate of ageing from maximum age records alone.

Methods of obtaining vertebrate data

It is difficult, but not impossible, to obtain actuarial statistics for vertebrates, and as they are virtually essential to any biologically directed attack on age problems, we should devote our attention to getting them, as a matter of urgency. They represent an expendable problem, moreover, since time once spent will not require to be re-spent later.

Age-mortality data can be obtained in three ways: (1) from populations of animals specially kept under close observation throughout life, (2) from analysis of existing records, (3) by cross-sectional studies which indicate the simultaneous mortality in each age group over one period of time, instead of the successive mortalities of the survivors of a cohort followed throughout life. The results of (3) will differ numerically from those obtained from the same animals by the first two methods if there is a secular change in mortality during the lives of the longest-lived individuals. With this method we should include cross-sectional studies of animals which can be aged by inspection, particularly the analyses of fish populations by means of catch curves (Ricker, 1948). The number of instances in which wild populations can be used for ageing studies is, however, so far small; although even in small birds whose mortality is substantially constant, ringing studies show that

more individuals reach old age than the early death rates would lead us to expect (Haldane, 1953).

It is evident that we can expect to obtain figures for most of the long-lived animals only from existing records. These include kennel and stud books, notes kept by laboratories or by amateurs, and the record files of zoological gardens. The material varies greatly in quality, and the statistical treatment which it requires is different from that which serves for laboratory or human actuarial work, since the data consist of

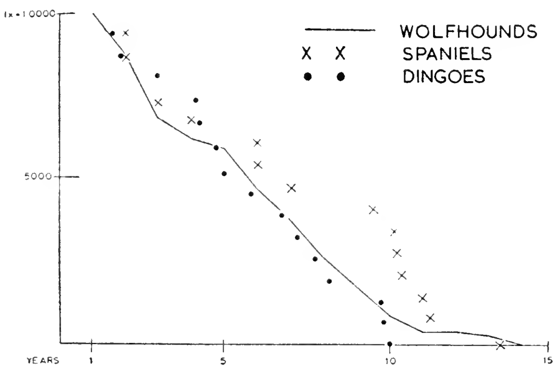


Fig. 2. Survival curve of 83 Irish wolfhounds by yearly totals, sexes combined (continuous line). Crosses (×) indicate ages of death of 14 spaniels from the same kennel, and points (●) ages of death of 15 dingoes in the London Zoo.

multiple small samples, and in most cases there are substantial losses from the record by sale, culling, or deliberate killing in the course of experiments.

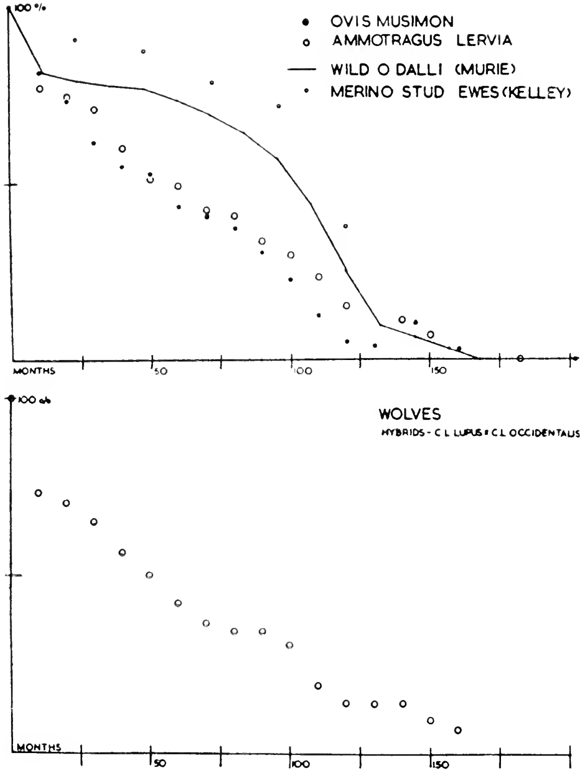
We have been collecting material from sources of this kind. It has so far been possible to construct survival curves for one breed of dog, the Irish wolfhound (Comfort, 1957) and for several animals (hybrid wolves, *C. lupus lupus* × *C. l. occidentalis*; dingoes; *Ovis musimon*, the mouflon; *Ammotragus lervia*, the Barbary horned sheep; *Dolichotis patagona*, the Magellan cavy; *Boselaphus tragocamelus*, the nilghaie; and *Muntiacus muntjac*, the muntjak deer) from the records of the

Zoological Society's collection in London. The choice of species in these cases was determined entirely by the number of scoreable lives available. In consequence the data are practically confined to species breeding readily in captivity and kept in considerable numbers. The wolfhound data were obtained from a kennel book which was exceptional in that the breeder had ascertained, and recorded, the subsequent fate of most of the dogs she sold (Fig. 2).

The most evident feature of the survival curves which we have obtained is their similarity. They all approach a straight line over the whole adult period, when survivorship and time are plotted on arithmetic co-ordinates. Such a distribution is unusual in comparison with the log-linear decline usually found in wild populations and the plateau of low adult mortality in fully domestic animals. It indicates (1) that the number of animals of a cohort which die in unit time is constant, (2) that the force of mortality rises continually with age, since each batch of deaths is an increasing fraction of the total surviving, (3) that the distribution of lifespans is rectangular, there being no commonest age of death after the initial infant mortality is past (Fig. 3).

The survival curves of the two species of sheep (*Ovis musimon* and *Ammotragus lervia*) and of the wolf × timber wolf hybrids in the London Zoo are practically identical. That of Grecian wild goats, also in the London Zoo, is closely similar in form, but declines at rather more than half the rate. The lifespan of domestic goats appears, from some initial figures which are not yet complete, to be very much shorter than that of wild goats. The figures we obtained for wolfhounds showed a large sex difference in mortality; they also indicate that there is little difference between age-mortality pattern in a cohort of highly inbred pedigree dogs and in dingoes, which are wild-type, probably feral, dogs.

In examining figures of this kind it is possible to see whether any of a population of animals has, in fact, reached old age by comparison with maximum age records. If we obtain a series of curves in which the survivorship can be followed through



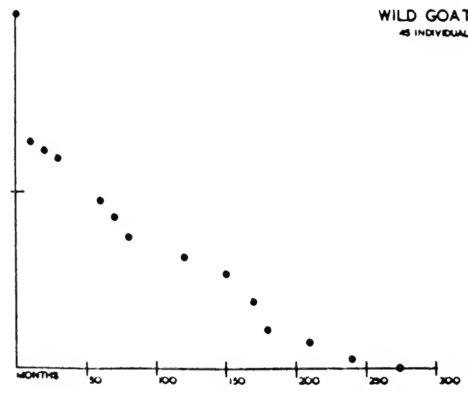


FIG. 3. Survival curves of animals in the London Zoo, sexes combined. Drawn from 158 mouflon sheep (*Ovis musimon*), 148 Barbary horned sheep (*Ammotragus lervia*), 95 hybrid wolves (*Canis lupus lupus* × *C. l. occidentalis*) and 45 wild goats (*Capra hircus aegagrus*). The curve of survival of wild sheep (*O. dalli*) and points representing the survival of Merino ewes, are superimposed on the survival curves of zoo sheep.

progressively better environmental conditions, it may be possible to use them as a source of information about the nature of the processes which operate in domestication. This is an important question in age studies, since in studying the gross loss of vigour which occurs late in senescence we are, in fact, studying a phenomenon which only rarely occurs in the wild state, and when we study it in circumstances which allow a high proportion of animals to reach old age we are, in fact, producing by domestication an organism different from that which arose in the first instance by natural selection. Life tables obtained from zoo populations represent a special biological situation, in which the animals receive sufficient protection from predators to enable them to reach old age, but are subject to higher mortalities from epidemics, parasites, and behavioural causes such as fighting due to the interruption of territory behaviour.

We can compare the performance of the sheep with that of Merino ewes, and also with that of the wild sheep studied by Murie (1944) who prepared a life table by ageing the skulls of animals dying in the Mount McKinley National Park. This is one of the few cases where senescence has been demonstrated in a wild mammalian population. The comparison is interesting for the much better performance of the wild sheep, even when extensively preyed on by wolves, compared with that of the zoo animals, but its main interest is in showing the relationship in time between the mean, median and limit of the two survival curves, since the percentages reaching extreme ages were similar in all three cases. Such information, and the comparison of longitudinal and cross-sectional studies under different environmental conditions, could be used to study directly the distribution of individual vitalities, or potential lifespans, in a population.

In other cases it is necessary to work with specially maintained populations. We have set out to obtain detailed vital statistics for one poikilothermal vertebrate, the guppy (*Lebistes reticulatus*), by observing the lifespans of several thousand specially kept individuals. We have found that this

fish exhibits a very characteristic increase in mortality with age, which we have been able to correlate with its growth pattern and reproductive rate under various experimental conditions. These experiments are still in progress. Under good conditions, at 23° C, less than 10 per cent of fish in a cohort have died before reaching 1 year of age, and less than 10 per cent exceed 1000 days in the series so far kept. The force of mortality in this species, both in the females, whose rate of growth depends on feeding and space, and the males, which cease growing soon after sexual maturity, approximates in its age distribution to that of the laboratory rat. We are collecting parallel figures for the rate of fin regeneration at different ages and under different conditions of growth. I quote this work as an example of the type of study in which it is possible to combine observational with experimental work and direct them to attack particular problems. Investigations of this kind must necessarily be confined to short-lived species, and our experience with *Lebistes* suggests that even with an unusually hardy and semi-domestic organism, and even in the absence of epizootics and accidents, to which such research is extremely vulnerable, the minimum time necessary to complete such a study is twice the life-time of the organism.

I have not, I hope, suggested in the course of this paper that fundamental studies of the processes which go on within organisms, or the cells of organisms, are either not worth studying or inaccessible to study. The brilliant work of Sonneborn's school on the senescence of clones in *Paramecium* is an indication of what can be done in such fields—an apt one, for my purpose, because decline in vegetative lines of *Paramecium* is an example of an effect which was long adduced as evidence of a fundamental biological law, the necessity to maintain cell vigour by sexual reproduction, and which turns out to be peculiar to ciliates, since it is apparently due to uneven distribution of the chromosomes of a polyploid macronucleus. We require a larger amount of comparative information because the greater our knowledge of the pattern and distribution of age effects, the less will be the risk of over-

generalizing the phenomena we find. It would be possible to obtain, within a reasonable time, a knowledge of the comparative rates of ageing in vertebrates as full as our knowledge, for instance, of their sexual cycles. This would require the collection and statistical analysis of as many suitable records as possible; it could be supplemented by cross-sectional surveys on domestic animals such as dogs, and possibly even on specially kept populations of the larger fish and birds. I think it is evident that if we could obtain vital statistics only for the main breeds of dog, the information would throw light on the effects, if any, of size on longevity, and of the endocrine and other differences found in pure breeds and in experimental crosses between them. Work on methods of ageing adult vertebrates, such as that described by Bryuzgin (1955) and used by Prof. Bourlière's colleagues on the ossification zones of snakes (Petter-Rousseaux, 1953) is clearly of great importance. I think that at present the most important requisite, however, is the co-operation of those biologists and others who have the data, or the opportunity to extract them, but who do not know the nature of the information wanted. I think a symposium similar to this one, attended by veterinary, stockbreeding and conservation workers, and directed to surveying available records would be of great value to the business of obtaining comparative vital statistics.

May I perhaps summarize the points I have made: our work is concerned with a series of phenomena arbitrarily chosen for study. They are probably similar in biological significance but they may be different in detail. The measurements and definitions which we use in comparative study must take this into account. For this reason it is convenient to base our comparisons, initially at least, on the decline of vigour with age as it is measured by the increase in the force of mortality: it is a prerequisite of any understanding of the evolution of this decline in man that we should obtain a better impression of its distribution in vertebrates, and we should devise ways of doing this as soon as possible.

Acknowledgement

I am indebted to Dr. L. Harrison Matthews, F.R.S., for permission to use the Zoological Society's records, and to the Nuffield Foundation for the research Fellowship under which this work was undertaken.

REFERENCES

BACON, F. (1645). Historia vitae et mortis. Dillingen.

BENEDICT, F. G., and TALBOT, F. G. (1921). *Publ. Carnegie Inst., Wash.*, **302**, 100.

BIDDER, G. P. (1925). *Nature, Lond.*, **115**, 495.

BIDDER, G. P. (1932). *Brit. med. J.*, II, 5831.

BOURLIÈRE, F. (1946). *Ann. biol.*, **22**, 249.

BRYUZGIN, V. L. (1939). *C. R. Acad. Sci., U.S.S.R.*, **3**, 403.

BÜRGER, M. (1954). Altern und Krankheit. Leipzig: Georg Thieme.

COMFORT, A. (1956). The Biology of Senescence. London: Routledge and Kegan Paul.

COMFORT, A. (1957). *Proc. zool. Soc., Lond.*, in press.

DICE, L. R. (1933). *J. Mammal.*, **14**, 147.

GARDNER, G., and HURST, H. (1933). *Proc. Utah Acad. Sci.*, **10**, 149.

GREEN, C. V. (1932). *J. Mammal.*, **13**, 45.

HALDANE, J. B. S. (1941). New Paths in Genetics. London: Allen and Unwin.

HALDANE, J. B. S. (1953). *J. Inst. Actu.*, **79**, 351.

HARVEY, G. F., and HEMS, J. (1948). The Goldfish. London: Batchworth Press.

HINSHELWOOD, C. (1951). *Nature, Lond.*, **167**, 666.

KELLEY, R. B. (1939). *Aust. vet. J.*, **15**, 184.

LESLIE, P. H., and RANSON, R. M. (1940). *J. anim. Ecol.*, **9**, 27.

LESLIE, P. H., TENER, J. S., VIZOSO, M., and CHITTY, H. (1955). *Proc. zool. Soc., Lond.*, **125**, 115.

MACLAREN, A., and MICHIE, D. (1954). *Nature, Lond.*, **173**, 686.

MACLAREN, A., and MICHIE, D. (1955). *J. Embryol. exp. Morph.*, **3**, 366.

MCCAY, C. M., MAYNARD, L. A., SPERLING, G., and BARNES, L. L. (1943). *Arch. Biochem.*, **2**, 469.

MEDAWAR, P. B. (1955*a*). An Unsolved Problem of Biology. London: Lewis.

MEDAWAR, P. B. (1955*b*). Ciba Foundation Colloquia on Ageing, **1**, 4. London: J. & A. Churchill Ltd.

MOLTONI, E. (1947). *Riv. ital. Ornithol.*, **17**, 139.

MURIE, A. (1944). The Wolves of Mt. McKinley. U.S. Nat. Parks Service.

ORR, R. T. (1939). *J. Mammal.*, **13**, 45.

PEARSON, K. (1897). The Chances of Death, and Other Studies in Evolution. London: Arnold.

PETTER-ROUSSEAUX, A. (1953). *Terre et Vie*, **100**, 175.

PITT, F. (1955). *Nature, Lond.*, **155**, 700.

√RICKER, W. E. (1948). *Indiana Univ. Publ.* (*Sci.*), **15**.
ROGERS, J. B. (1950). *Anat. Rec.*, **106**, 286.
RUDZINSKA, M. A. (1952). *J. Geront.*, **7**, 544.
RUDZINSKA, M. A. (1955). *J. Geront.*, **10**, 469.
SMITH, M. (1951). The British Amphibians and Reptiles. London: Collins.
SUMNER, F. B. (1922). *J. Mammal.*, **3**, 79.

[Discussion of this paper was postponed until after the paper by Prof. Bourlière.—EDS.]

THE COMPARATIVE BIOLOGY OF AGEING: A PHYSIOLOGICAL APPROACH

FRANÇOIS BOURLIÈRE

Centre de Gérontologie Claude Bernard, and
Laboratoire de Physiologie, Faculté de Médicine, Paris

PHYSIOLOGICAL changes occurring after maturity have been mainly studied in man and a few laboratory mammals, such as rats, mice and guinea pigs. The main results of these investigations have been reviewed elsewhere and another summary would seem to be superfluous. It is sufficient to say that most of these changes lead to a decrease in viability and an increase in vulnerability of the organism. The morphological and functional involution which characterizes ageing starts in the various organs at varying periods early in life. Its end-result at the organismic level is always a decreased adaptation to the environmental conditions.

But senescence is not limited to mammals, and one may wonder if the ageing patterns of lower vertebrates and invertebrates are altogether similar to that of man and his laboratory animals. To be sure, our knowledge of the ageing processes in the various types of animals is hardly more than sketchy, but some comparisons are nevertheless possible. We have, for instance, some indications of the influence of age on fertility of various warm-blooded and cold-blooded vertebrates. In the rat, the number of young per litter is maximal at the second or third litter and decreases regularly afterwards (King, 1924). In guinea pigs, the greatest fertility coincides with early maturity (211–420 days) and then diminishes (Rogers, 1951). The highest egg production in fowls, as shown in Fig. 1, is observed in young birds, and the number of eggs laid annually decreases regularly as females grow older (Romanoff and Romanoff, 1949).

The situation is quite different in lower vertebrates. The fertility of the females does not generally lessen with age, and the longer and older the female, the greater is its capacity to produce eggs—at least up to a certain age. In snakes, for instance, fertility increases with the age of the mother. In the *Natrix natrix* we have studied in our laboratory, the clutch

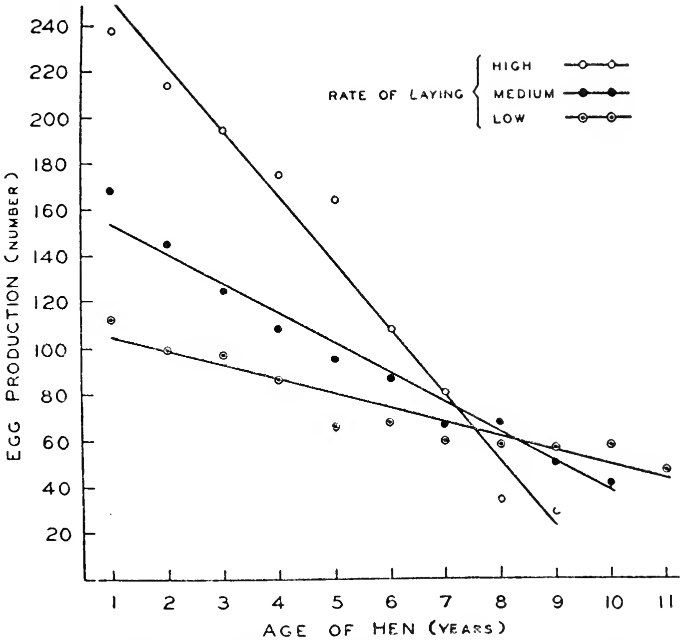


FIG. 1. The variation with age of egg production in three strains of domestic fowl. (After Romanoff and Romanoff, 1949.)

size varies with age from 16 to 51 eggs per clutch (Fig. 2). Another species, *Coronella laevis*, shows the same trend (from 4 to 13 young per brood in that viviparous snake). A senile decrease in fertility can nevertheless be noted in the oldest individuals, as shown by Klauber's (1936) data, but it takes place at a much later age than in warm-blooded vertebrates of similar size (Fig. 3). In fishes the situation appears to be quite similar. To take a single example, the average egg

production per female and per year of North Sea haddocks was found by Raitt (1932) to be 31,000 eggs for the second year of life, 159,000 eggs for the fourth year of life, 278,000

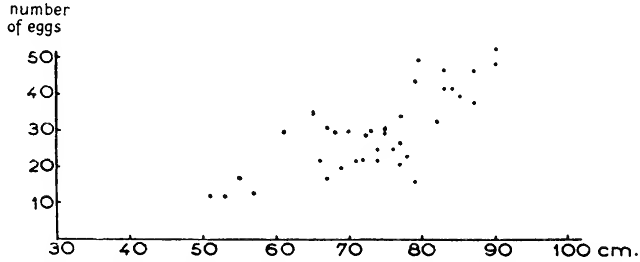


FIG. 2. The variation with age of egg production in a snake, *Natrix natrix*.

eggs for the sixth year of life and 3,295,000 eggs for the eleventh year. As in snakes, there is nevertheless some evidence of a slight decline in egg production in the oldest females. In small and short-lived species there is also some

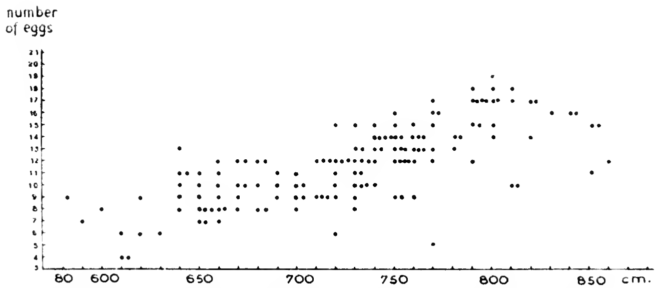


FIG. 3. The variation with age of egg production in a snake, the Californian rattlesnake. Note the difference in the pattern of reproductive senescence between the snakes and the fowl. A senile decline in egg production in longest (i.e. oldest) females is nevertheless perceptible in rattlesnakes.

indication of reproductive failure with increasing age, but accurate figures are unfortunately lacking. However, the difference in the pattern of reproductive senescence between warm-blooded and cold-blooded vertebrates remains obvious

and it is quite probable that similar differences exist in the rate of ageing of most organs and functions. What is already known of the disparities in growth pattern and in comparative incidence of certain degenerative diseases in both groups supports that idea.

As the various kinds of animals, even among vertebrates, do not age in the same way, comparative biology of senescence should help in distinguishing between the basic processes and modifications which are merely idiosyncrasies. When one considers, for instance, the unequal distribution of atherosclerosis among the various vertebrate groups, one is compelled to admit that the rôle of such a degenerative process in ageing phenomena has been greatly overestimated. I myself have dissected many old fishes, reptiles, passerine birds, rodents and even primitive lemurs without finding much conclusive evidence of atherosclerosis.

More important than that, the comparison of age changes in various phyla of the animal kingdom should help us to isolate their "common denominator". Besides the usual laboratory animals—and the senile hospital patient—the experimental gerontologist should certainly take advantage of a closer study of some "natural experiments" which could seldom, if ever, be performed under laboratory conditions.

To illustrate that point, I should like to recall a few observations and experiments which seem to indicate some correlation between the rates of energy-turnover and ageing.

Fifty years ago, Rubner (1908) was the first to propose a definite relationship between rate of metabolism and duration of life. He believed that various mammals during their lifetimes used up approximately the same number of calories per unit of weight. As the smaller species have a much higher rate of metabolism per unit of weight than the larger forms, in order to preserve a constancy of body temperature, their duration of life should accordingly be shorter than those of bigger species, and such is actually the case. Rubner did not furnish any experimental evidence to support his idea, and his theoretical work was even challenged by some authorities.

During the twenties, Pearl (1928) and his associates were nevertheless able to establish, by careful experiments, that the lifespan of some invertebrates could be condensed or lengthened as the average metabolic rate was raised or lowered. Without considering all the details, it seems timely to recall their main observations. MacArthur and Baillie (1929) kept parthenogenetically produced *Daphnia magna* at various temperatures, ranging from 8° to 28° C, the other conditions remaining constant. In this way the duration of life of these cladocerans varied widely, the mean lifespan being, on an average, 25·5 days at 28° C as compared with 108·1 days at 8° C. At the same time they found that an acceleration of metabolic rates was always associated with a nearly proportionate decrease in duration of life. An elevation of temperature from 8° to 18° C increased the *Daphnias*' heart-rate by 412 per cent and their susceptibility to KCN by 435 per cent, whereas it shortened their lifespan by 423 per cent. Similar results were obtained in Japan by Terao and Tanaka (1930) on another cladoceran, *Moina macrocopa.*

Using the fruit fly, *Drosophila melanogaster,* Alpatov and Pearl (1929) came to very similar conclusions when studying the effect of the temperature, during development and imaginal life, upon the duration of life of that insect. A high temperature (28° C) during development shortens the duration of the subsequent imaginal life at all temperatures and in both sexes, with but one exception; e.g., females reared at 28° C had a mean duration of life of 28·5 days, while those reared at 18° C lived for 70·6 days. The effect of temperature during the imaginal stage upon the duration of life of the imago itself is equally striking; as the environmental temperature is higher flies live, on an average, a shorter time. Similar results have been obtained by Loeb and Northrop (1917).

Another means of influencing the metabolic rate of invertebrates is food restriction. Using the two already mentioned species of cladocerans, Ingle (1933) and Ingle, Wood and Banta (1937) have found that *Daphnia* which are starved live, on an average, about 40 per cent longer than those which are

well fed, while their metabolic rate, as indicated by the heart beat frequency, is significantly lower than in normally fed individuals. Kopec's (1924) experiments with caterpillars of *Lymantria dispar* gave similar results. Among rotifers the lifespan of the adults seems likewise to be inversely proportional to metabolic rate. Encysted adults may survive as long as 59 years in diapause (Rahm, 1923), while most active individuals live only a few weeks.

Besides these experiments, numerous field observations appear to confirm both the influence of temperature and the effect of food restriction and reduced activity on the duration of life of invertebrates. To take but a few examples, a tropical butterfly studied by Fountaine (1938) in Cambodia reached its imaginal stage in only 7 days, while some Arctic Satyrids need more than 2 years to reach the adult stage. Among some molluscs living in the same area, growth rate, ultimate size and duration of life seem to depend on the amount of food available in the various environments. Such appears to be the case, at least, for the limpets observed by Fischer-Piette (1939) in Brittany.

Thus, it seems quite certain that the rate of energy metabolism has something to do with the duration of life in invertebrates, but one may wonder if such a correlation exists in vertebrates, especially in those groups whose internal milieu is kept at a constant temperature by efficient thermoregulating mechanisms. McCay's experiments (McCay, Crowell and Maynard, 1935) have shown that it is possible to lower the energy-turnover of rodents by supplying them with the least possible amount of food, and consequently to almost double their lifespan—a fundamental discovery which has been confirmed many times. But many other facts seem to indicate the major influence of the metabolic rate on the ageing processes of vertebrates.

Fishes offer remarkable opportunities for study from that point of view. Large samples of populations living in definite environments can be easily collected and an accurate determination of their age-composition can be made in most cases.

On the other hand, some species have a rather wide geographical distribution and live in waters whose temperature and food-content vary greatly.

In such conditions striking differences in growth rate, tempo of life and longevity can be found. To choose but one example, the comparison of the data of Brown (1943) and of Miller (1946) on two different populations of the North American grayling (*Thymallus signifer*) seems to me highly suggestive. The Michigan subspecies has a very rapid growth and matures early, but has a short life. The oldest individual seen by Brown was in its sixth year of life. On the other hand, the Arctic subspecies found in Great Bear Lake in northern Canada does not reach its sexual maturity before its fifth summer; it attains a greater ultimate size and also a much greater longevity. Some specimens caught by Miller were in their twelfth summer. The length of life of the Arctic population is therefore about three times that of the southern one.

Similar instances could certainly be found easily in ichthyological literature. Sticklebacks (*Gasterosteus aculeatus*) do not live longer than 14 to 18 months in France, but require several years to reach their maturity in more northern latitudes. Pilchards (*Clupea pilchardus*) are said to have a slower growth and a longer life in the English Channel than off Saint Jean de Luz and the Spanish coast.

Similarly, the duration of the larval period of some widely distributed amphibians varies greatly with latitude. In North America, for instance, according to Oliver (1955), the bullfrog (*Rana catesbeiana*) undergoes metamorphosis at the end of its first winter in sunny Louisiana, while in the extreme northern part of its range (Nova Scotia) it may spend three winters as a tadpole. Unfortunately we have no data on the comparative longevity of adults in both cases.

Among lizards, striking latitudinal differences in growth pattern and lifespan are known. In Florida, for instance, 94 per cent of the southern fence lizards (*Sceloporus undulatus undulatus*) were found to live less than one year, and none lived in their natural environment for two years. This warm

climate permits this lizard's population to be actually active every month of the year and individuals grow throughout their short lifespan. On the other hand, the northern race of the same species (*Sceloporus undulatus hyacinthinus*) usually lives for more than 4 years in Maryland, probably reaching a maximum age of 8 years. In this colder climate the fence lizard is active only seven months of the year and its growth appears to stop after about the fifth or sixth year. In this case growth thus appears to be determinate in one part of the animal's range and indeterminate in another.

All these observations concern poikilothermal vertebrates, and one may wonder if something comparable may occur in homeothermal animals. Recent ecological studies suggest that such may be the case. The efficiency of thermoregulating mechanisms varies greatly in the various groups of birds and mammals. Some species regularly enter periods of hibernation and of estivation during which their internal temperature may fall to a point close to that of the environment. In such a state the metabolic rate is greatly reduced, often during many months a year. Others, like bats, normally have a very low metabolic rate, almost at the hibernating level, during about 20 hours a day, their oxygen consumption increasing only during flight. Such a periodic reduction of the energy metabolism, so well studied by Pearson (1947), is not at all restricted to the growth period, but continues throughout life. It seems to me highly significant that these mammals which have a poor temperature regulation and a normally low rate of metabolism are precisely those whose lifespan is far longer than that of other mammals of similar size. In 1947, I reported the case of a wild *Rhinolophus hipposideros* banded as adult in October 1938 and still living in fine condition in May 1946. More recently, Dorst (1954) reported the cases of marked *Rhinolophus ferrum-equinum* and *Miniopterus schreibersi* having reached 15 and 14 years respectively in wild conditions, potential longevities of 8 to 12 years being very frequent in these bats. Cockrum (1956) has even more recently published additional observations for North American species which

confirm European banding results. It thus appears that in these mammals a reduced energy metabolism and a low fecundity is definitely associated with a long lifespan. Inversely, shrews with their high metabolic rate and fecundity are probably the shortest-lived mammals, especially dwarf species, like *Suncus etruscus*, whose adult weight seldom goes beyond 2·5 g.

The study of age-changes in those short-lived animals would certainly be greatly rewarding, and we are now trying to raise them under laboratory conditions. Temporary poikilothermism, seasonal or diurnal variations of metabolic rates and long duration of life seem likewise to exist in some birds, like swifts, hummingbirds and goatsuckers (Pearson, 1950, 1954; Jaeger, 1949), and even in some primitive primates (Bourlière and Petter-Rousseaux, 1953; Bourlière, Petter and Petter-Rousseaux, 1956).

Furthermore, the comparative study of age changes should not be limited to the various zoological groups, but should be extended to the various human populations, especially those whose genetic constitution, nutritional status and ecological environment is so different from our West European or North American standards. When teaching at the University of Hanoi, Indochina, I was greatly impressed by the way most Tongkinese peasants age, as compared with their own countrymen living in the big cities—and even more with westerners. On the other hand, the so-called "premature ageing" of some "primitive" populations would need to be investigated before it is too late. I know that such studies are most difficult to carry out. Even if laboratory facilities were available, it might be hard to get an accurate knowledge of the actual age of the subjects examined. Furthermore, any study of that type needs to be completed by a careful anthropological and nutritional survey of the population sample which is investigated. There are, nevertheless, some countries like India, Indochina, Mexico and the West Indies, where such studies could be made.

To sum up, one may conclude that functional age changes

can be found in any kind of animal and that senescence is thus a fundamental characteristic of all living matter. But these various kinds of animals apparently do not age in the same way and some groups constitute, from the physiological point of view, natural experiments on a grand scale, which should be studied more closely. The understanding of the normal ageing processes in man should certainly benefit from such comparative studies.

REFERENCES

ALPATOV, W. W., and PEARL, R. (1929). *Amer. Nat.*, **63**, 37.
BOURLIÈRE, F. (1947). *Mammalia*, II, III.
BOURLIÈRE, F. (1954). Josiah Macy Jr. Foundation, Trans. 15th Conf. on Problems of Ageing, p. 126.
BOURLIÈRE, F., PETTER, J. J., and PETTER-ROUSSEAUX, A. (1956). *Mem. Inst. Sci., Madagascar*, Ser. A, **10**, 303.
BOURLIÈRE, F., and PETTER-ROUSSEAUX, A. (1953). *C.R. Soc. Biol., Paris*, **147**, 1594.
BROWN, C. J. D. (1943). *J. Wildlife Mgmt.*, **7**, 353.
COCKRUM, E. L. (1956). *J. Mammal.*, **37**, 48.
COMFORT, A. (1956). The Biology of Senescence. London: Routledge and Kegan Paul.
DORST, J. (1954). *Mammalia*, **18**, 231.
FISCHER-PIETTE, E. (1939). *J. Conchyliol.*, **83**, 303.
FOUNTAINE, M. E. (1938). *Entomologist*, **71**, 90.
INGLE, L. (1933). Science, **78**, 511.
INGLE, L., WOOD, T. R., and BANTA, A. M. (1937). *J. exp. Zool.*, **76**, 235.
JAEGER, E. C. (1949). *Condor*, **51**, 105.
KING, H. D. (1924). *Anat. Rec.*, **27**, 337.
KLAUBER, L. M. (1936). *Occ. Pap. S. Diego Soc. nat. Hist.*, **1**, 2.
KOPEC, S. (1924). *Biol. Bull.*, **46**, 1.
LOEB, J., and NORTHROP, J. H. (1917). *J. biol. Chem.*, **32**, 103.
MACARTHUR, J. W., and BAILLIE, W. H. T. (1929). *J. exp. Zool.*, **53**, 221, 243.
MCCAY, C. M., CROWELL, M. F., and MAYNARD, L. A. (1935). *J. Nutr.*, **10**, 63.
MILLER, R. B. (1946). *Copeia*, 227.
OLIVER, J. A. (1955). The Natural History of North American Amphibians and Reptiles. Princeton: Van Nostrand.
PEARL, R. (1928). The Rate of Living. New York: Knopf.
PEARSON, O. P. (1947). *Ecology*, **28**, 127.
PEARSON, O. P. (1950). *Condor*, **52**, 145.
PEARSON, O. P. (1954). *Condor*, **56**, 317.
RAHM, M. P. G. (1923). *Z. allg. Physiol.*, **20**, 1.

RAITT, D. S. (1932). Fishery Board for Scotland, Scientific Investigations, I.

ROGERS, J. B. (1951). *J. Geront.*, **6**, 13.

ROMANOFF, A. L., and ROMANOFF, A. J. (1949). The Avian Egg. New York: Wiley.

RUBNER, M. (1908). Das Problem der Lebensdauer und seine Beziehung zu Wachstum und Ernährung. Munich: Oldenburg.

TERAO, A., and TANAKA, T. (1930). *J. imp. Fish. Inst.*, **25**, 67.

DISCUSSION

Parkes: Dr. Comfort's paper brings up the old problem of why in some species one sex lives longer than the other. You showed that the female wolfhound lives longer than the male, so to that extent the wolfhound is similar to man. Are there any other species known to you where that happens?

Comfort: It seems to be very nearly general, not only in such mammals as have been investigated, but in a great number of other animals as well. The suggestion was made at one time that the longevity of the female depended on her being the homogametic sex, and that it ought to be the other way round in birds and butterflies, but as far as I know the results so far do not bear that out: in some butterflies the female is longer-lived, in others the male. In birds, at any rate in poultry, greater vigour of the female seems to be the rule. I don't know whether that is true, but it has been claimed.

Parkes: So that is a fairly general phenomenon for which there is no general explanation?

Comfort: Darwin gave the best "explanation" for it, when he said that it was a natural peculiarity due to sex alone!

Parkes: That is a description, not an explanation!

Verzár: The "biological age" in individuals as they get older in time is extraordinarily different. I think that your great variations, Dr. Comfort, may be explained by the fact that there are some individuals who are biologically still young, while others are already very old. The same appears from your survival curves; at least in many of your curves there seems to be some similarity to the Z-shaped survival curve in humans. There is, of course, an initial quick decrease in the first years of life, but if you cut that out, the curve is first more or less flat, and then it starts sinking in a Z-shape. This we see in man, in rats and, as you have shown us, in many other species. Can you explain why in rats, inbred for 25 years, we get this life survival curve?

Comfort: I cannot offer an explanation of it; I am still trying to arrive at a description of it. So far as the Zoo populations were concerned, we were not getting so much a Z-shaped curve as an arithmetical straight line, but it is clear from the behaviour of the sheep that this is due to the life not being so good in Zoos. If we were to keep them under really good conditions and if we were to choose strains that are hardy, we should expect to get a Z-shaped curve with a plateau of varying length. We get the effect in guppies where there may be virtually no deaths before

the end of the first year and very nearly the whole population die by the end of the third year. What the explanation of that is, I don't know. I think we must try to find a way of measuring the dose of life which will prove fatal to any given individual, before it has actually proved fatal to him.

Olbrich: Do you know from which diseases your animals suffer or suffered, Prof. Bourlière, and for how long? Do you know the frequency of illness in your animals? In human beings we do know. Do you include all diseases or illnesses interfering with lifespan and, perhaps, also with processes of ageing? The process of ageing is a doubtful business altogether. Until now neither biologists nor scientists could present any evidence saying: "These are the signs and symptoms of ageing only and not of a disease." For example, the low basal metabolic rate is a very important and interesting subject, and every year different results are published on it. Nagorny, in 1939, published the results of his investigations on people aged between 90 and 100 and found that they had normal basal metabolic rates, normal blood sedimentation rates and so on—in fact, all findings were normal. Now, if it is true that humans with low basal metabolic rates live longer, then in myxoedema they should survive longest, but unfortunately the clinical experience does not bear this out. Another important point is that you can prolong the life of patients by starving them. We do this in our clinic; I do it to myself as well. Now you advise me to put them into ice-cold temperatures—and that is very difficult for me—and to let them sleep for twelve hours!

I do not understand, firstly, what factors of disease influence these vital statistics or these curves in the excellent studies of both Dr. Comfort and Prof. Bourlière, and, secondly, whether one single factor—either basal metabolic rate or food restriction—has anything to do with prolonging life or influencing resistance to disease.

Bourlière: The data on the comparative incidence of various infections and degenerative diseases in the different zoological groups are so scarce that it would be dangerous to generalize. Some studies have been made which tend to show that, in mammals, arteriosclerosis is more common in ungulates and in some carnivores, whereas it is quite rare and even absent in most rodents, insectivores and bats. In birds the same disease seems to be more common in birds of prey than in passerines. The cause of these differences remains unknown, but the rôle of the dietary differences is probable. With regard to cancer, we are better documented, but the gaps in our knowledge are still very numerous. It has long been said that cancer does not occur in fishes and reptiles, but that is wrong. Numerous observations have shown that certain types of cancer may be observed in those animals. But one must not forget that in natural populations very few individuals actually die from disease; most die from accidents, mainly from predation and parasitism.

Olbrich: Disease is also an accident!

Bourlière: Yes, infectious diseases are a kind of parasitism very poorly known in "lower" vertebrates. But their rôle as a mortality factor is probably less important in natural populations than in domesticated ones. Recent ecological studies on wild bird and rodent populations

have emphasized this point. As for degenerative diseases, their rôle is negligible; their importance as a mortality factor in populations is really a by-product of domestication (in animals) and of civilization (in man).

Olbrich: The point is that you don't know the diseases. We know the human diseases, we know what humans die of, we have the postmortems. Your wild animals have various diseases that you don't know; you have no postmortems. So your vital statistics are not the vital statistics we deal with. Ageing must be something you can define and you, as a scientist, must be able to tell me as a clinician, "These are the clinical signs of ageing." And it must not be a disease; once it is a disease it is no longer "ageing" but "accident of death". That is all right, the mortality rate with respect to longevity, I accept that.

Lorge: This point about variability being raised here is very interesting. I have been trying to estimate whether ageing brings greater or lesser variability. You look at a trait like vision and you find diminished variability with age, but look at vigour and you find augmented variability. For an overall factor like vigour, subject to a heterogeneity of causes, you get wider variability. The methodological issue is whether or not multiple causes can be summarized simply into a gross trait leading to the conclusion, "Here we have an increase in variability." The probabilities are for an increase in variability, due to the fact of two kinds of circumstance : one is disease, accident or special population, and the other is the factors related to vigour. Now, those that are related to vigour (with increasing age) tend to lead toward diminished variability. In human populations we lack knowledge about the extremes of the range, i.e. the people who are extraordinarily vigorous or who are extraordinarily non-vigorous. They are the people we do not test, we cannot get them to be examined.

Olbrich: Even accepting what you say about vigour, more vigour or less vigour is a clinical sign.

Lorge: I agree.

Olbrich: That is the reason for your great increase in coefficient of variation. This signifies that the shape of Dr. Comfort's blood-pressure curve * consists of two types of people, the pathological and the normal, mixed together as one population.

Lorge: There is actually a third population. This is very much like the analysis of the life tables made by Arne Fischer years ago. He suggested that difference in variabilities may be a function of two, three or four sets of causes. I think his point raises the question of methodology very strongly.

Baló: I agree with Dr. Olbrich that in studying the lifespans of different animals we need to know their diseases. I studied recently arteriosclerosis in the dog. This research was undertaken because, following the work of Anitchkow and co-workers, the rabbit has been the animal generally used in studying arteriosclerosis and it has been suggested, especially by American workers, that we should use some other animal, for instance the dog, which is more like the human. In studying this condition, I found that there is a very common parasitic disease of the dog,

* Not submitted for publication.—EDS.

called spirocercosis. This disease is localized in the arteries. I have read in the literature that it is very common in the Soviet Union and common in parts of Europe, while it is less so in Western Europe; I have seen no publication from the United States on this condition. If, therefore, we study the diseases of animals, we can explain the cause of death and we can then distinguish some processes which are not due to ageing but to disease.

Landowne: On the subject of variability, if there are multiple causes which all lead to changes in one general direction with age, absolute variability can be unchanged or even reduced; where data show an agewise diminution, the variability may appear to increase if expressed as coefficient of variability or if measures of technical variability are involved. In looking for this increase in variability with age in human populations we have seen all three things: increase, no change and decrease. Consequently, I wonder whether this expression of variability does not depend upon the point of view you take—in the case of the gonadotrophin study as an index of endocrinological change—whether in each instance it may not have its own explanation rather than being a fundamental biological process.

Lorge: The issue is more complicated than that. If the gross function which is death, is multiple cause, then the query is: what is the relationship between means and their variabilities and the correlations among the traits that are involved? Any sort of variability can be produced by hypothesizing what are the correlations and the means. If the means are negatively associated, they give one kind of a picture. If you discuss variability in ageing, the question then becomes one of isolation of the relative differential components, whether factorially or in any other way; and I think, at the present moment, even in biological functions such as blood pressure, as you have pointed out, we don't even know what all the components are. I was greatly impressed by the fact that the blood pressure data here reach to ages 67 or 68. In the United States much effort and money have been expended in trying to get data to the hundreds. It really is most difficult data to collect. We do not know what happens after age 67; and, with the increase in longevity, let us say of Western European and North American populations, the problem of getting people to subject themselves to blood-pressure tests becomes a problem of data collection too. All in all, the problem of making an assertion about variability depends on a methodological issue of the isolation of the components underlying a particular overall trait, be it death or blood pressure.

Gillman: There are certain issues arising from Prof. Bourlière's paper on which I think some contribution can come from South Africa. He made the statement that he believed the death of many species, such as lizards and reptiles, was usually accidental. I cannot agree with this. Some years ago, by chance, a student of mine at the University of Witwatersrand, in investigating the haematology of reptiles, found, among other very interesting things, an incidence of parasitic infections so high that up to 80 per cent of one particular species of lizard in the wilds were afflicted by haematological gregarine parasites. Similar observations were also made in certain snakes, again caught in the wild. All these

animals were caught by this young man, Dr. U. de V. Pienaar, and then brought to the laboratory for study. A reptilarium was started in Johannesburg, and a number of lizards have now been studied for several years. This high incidence of parasitic infection in these animals was regularly associated with very profound anaemias which apparently contributed to their deaths. Frequently, too, Dr. Pienaar found, during his excursions into the wilds, a number of animals that had apparently just died and in blood smears from these lizards he also found anaemias which were very profound. Full postmortem examinations (macroscopic, histological and haematological) of these animals also revealed evidence of gross anaemia. So Prof. Bourlière's statement certainly does not seem to hold true for our country.

Another point arises from the discussion relating to nutrition. There is a tendency in the nutritional literature to emphasize the caloric values of diets, and in the light of recent work emphasis is now being placed on the fat content of the diet. I think, however, that other features of the qualitative aspects of the contents of natural human diets, combined with quantitative defects in the diet cannot be overlooked. The need for studying nutrition in disease or for doing nutrition surveys has, of course, been mentioned. However, one realizes more and more, by being in contact with populations in Southern Africa—not just Johannesburg or Durban, but in field work as well—that unless thorough studies are made, not only of the nutritional state of the people at any particular time, but especially of the nutritional habits of the people from birth, one tends to get a completely distorted picture. The fact is, at least in the Union of South Africa, that the African people are frequently malnourished, virtually before birth, and they are malnourished, qualitatively and quantitatively, certainly from birth until their premature deaths. In my opinion, based on some 14 years of study of these people, it seems that the long-term effects of the overall pattern of chronic malnutrition among these people on the disease incidence among them, cannot be overlooked. One of the characteristic findings in adults (fairly young adults in terms of Western concepts—aged 30–40) is the evidence of lowered basal metabolic rate and clinical signs of a myxoedema-like picture. Also of interest are the changes in blood pressures observed in cases that we have now followed through repeated admissions to hospital with episodes of acute nutritional failure (pellagra) superimposed upon chronic nutritional failure. At one stage they manifest high blood pressures, both systolic and diastolic, and then, with the progress of the underlying chronic nutritional failure—frequently accompanied by the onset of severe liver disease—the blood pressure drops progressively and they are admitted to the hospital with blood pressures of about 90 or 100 systolic, and diastolic pressures well within the range of so-called "normal" young people.

Bearing all these things in mind, I think that Dr. Olbrich's remarks are very pertinent. Ageing in an African population cannot be effectively studied, in my opinion, without a thorough appreciation of the overall life pattern of the population being investigated.

Bourne: The comments that have been made about nutrition in human

populations might well be applied to some of the animal studies that have been described. Dr. Comfort, in producing some of his Zoo figures, explained to us the difficulties of assessing and interpreting this type of material, but I should imagine that the vast majority of animals that live in Zoos are being badly nourished from a qualitative point of view, at least. Recently, we (The Nutrition Society) have attempted to arrange with the Zoological Society a combined meeting during which we hoped to discuss the nutrition of animals in captivity, but the Zoo people pointed out that they could only say what they gave to animals; they could, in fact, contribute nothing as to the nutritional requirements of any of them. Taking as an example one particular group of animals, the carnivores, it is almost certain that these animals are grossly deficient, particularly in the vitamin B complex. They have a short digestive tract, the food moves fairly quickly through it, there is probably very little opportunity for bacterial synthesis of B-complex vitamins, and it seems highly probable that all animals in that group are in a subnutritional state as far as these vitamins are concerned. This applies probably to domestic dogs also; I have seen some spectacular improvements in health and well-being of dogs after they have been fed their extra supplements of dried yeast; as you know, most carnivores when in the wild eat a whole animal, the liver and kidneys and so on, not just the flesh which is all they are given when they are kept in Zoos or domesticated in the home. I have seen a remarkable improvement in a non-carnivore, namely a race-horse which, at one stage, was about to be condemned to death because of its very poor condition. Within three months of being given regular vitamin B complex in the form of dried brewers' yeast it was winning races without, I might say, my money being on it! The significance of these comments is that longevity statistics for animals kept in Zoos and kept domestically are affected by the almost certain state of sub-optimal nutrition in which these animals live.

Olbrich: This is not strange. We have to examine the nutrition but we also have to examine the effect of nutrition on the animal or human being. For example, the specific dynamic action of proteins diminishes the older a person gets, and this is a very important point. Perhaps this applies to animals too; we must not only examine feeding but the response to feeding.

Landowne: Prof. Bourlière, I think you have outlined the opportunities for experiments of this sort. Tadpoles that appear to have different rates of maturation at different temperatures may do so because of environmental factors other than thermal factors, and the experimental approach could be applied to test such a possibility. For example, their food supply may be quite different too. And in another category, the wild goat story would be even more interesting if one could anticipate the early drop in the slide that you showed, Dr. Comfort. Have we not here the possibility of asking questions which might bear on the mechanism of these observations? Can you do something in the London Zoo towards modifying the nutritive environment of these animals? I mean, cannot one suggest that they feed them in two ways rather than in one way over any given period?

Comfort: They would not have enough of them at any time to make a significant experiment; that is the difficulty. Assuming this goat figure turns out not to be merely an accident of this particular population, since this is a fairly small group, if we find the same in other goats it might be even more interesting to see whether this is due to any difference, as Prof. Bourlière suggested, in their metabolic rates. And it might be interesting to see whether it is in fact true that the more inbred domestic goats decline at a greater rate.

Landowne: With regard to Prof. Gillman's very interesting report on the parasitism of lizards, we see many old people with extensive atherosclerosis and other disorders who live with their infirmities and disease. Does this anaemia or this parasitic infestation account in the main for the mortality of these animals? These questions that seek beyond the first line of evidence sometimes yield most discouraging results. But this, to me at least, opens the pattern of investigation : you make the observation, you then look for the experiment.

Gillman: As I mentioned, after Dr. Pienaar's initial findings (recently reported fully in his Ph.D. thesis—and which were fascinating from a number of points of view, including the *types* of anaemia which develop in these animals), a reptilarium was started in the Medical School. Quite a number of animals even bred in captivity. One variety of lizards, even in captivity, frequently succumbed to heavy gregarine infections. One of the biggest difficulties was to eliminate the apparently tick-like animal which infested the group and which was shown to transmit this infection. When the transmitter was eliminated the mortality rate of the young and of the middle group of animals, of about 6 or 8 months of age, decreased considerably. There did seem to be a definite relation, therefore, between infection with these blood parasites and mortality rates.

Best: Have you a good supply of lizards that are free from this infection?

Gillman : That I cannot say, unfortunately, because both my student and I have left the Medical School in Johannesburg. However, Dr. Pienaar has now been appointed to the position of game warden in one of the South African game reserves, and one hopes to get some fascinating information from him in the next few years.

Lorge: I would like to underline these comments that have been made about nutrition in another way : I have been interested in the supplementation of animal feeds with antibiotics, and am raising the general question "Whether any veterinarian (or any other related group) knows what happens to animals fed antibiotics as a supplement to prevent infection, as opposed to those given adequate quality and quantity of vitamins and the like, in terms of longevity and other traits?" I have seen no data, except that the group given antibiotic supplement is subject to less disease and comes to full size at an earlier age. It seems to me that here we have a series of interacting forces, not only caloric content and vitamin content but also the possibility of throwing off disease by a supplementation factor of some kind.

Bourne: There is some evidence that some of the antibiotics have a vitamin-like action.

Lorge: That may be so; it may be working both ways.

Kallmann: Dr. Comfort, you said that the ability of many animal species to reach old age is largely a function of domestication. Do you believe that domestication has a favourable or an unfavourable effect upon the ability to have an extended lifespan? Are corroborative data available?

Comfort: The animals we observed in the wild may, in some cases, reach old age; there are some species that appear to do so, but a great many do not. If we take, say, robins or rats, the proportion that live to old age is relatively small; in the case of rats it is virtually nil, in the case of robins, surprisingly enough, ringing studies show that you do occasionally get an 11-year-old or even 14-year-old robin in the wild. Now, when you wish to study these creatures and to have a statistically significant proportion reaching old age, you have to bring them more or less under your control in the laboratory, and in doing so you automatically produce what is effectively a different organism. It is almost impossible to avoid doing this, particularly if you are breeding over a number of generations: you find that you are exerting various unrecognized forms of selection. This is true in the case of *Drosophila*, the fruit fly, which breeds rapidly. If you bring in the progeny of wild animals, you yourself will change as you learn better and better ways of keeping them, and they tend to change due to selection and to the influence of the various new factors to which they were not exposed in the wild. If you prevent the less vigorous of them being eaten by wolves or caught by ferrets in early life, you may find that those less vigorous individuals will live on and develop diseases or die at another stage in their development; the animal that has been in domestication for any length of time is really a rather different organism from the animal which exists in the wild. In order to produce circumstances in which many of these small rodents and birds can age at all or can age with any frequency, it is necessary to deal with the domestic forms. That is why I say that if you talk about the ageing of them in the wild, you are in general talking about a hypothetical state which neither evolution nor the actual facts of the matter have ever envisaged, and which we are not warranted in assuming.

Kallmann: I am just wondering why we still have so many non-domesticated rats.

Comfort: I think you will find that there are very few non-domesticated older rats. It would be interesting to know that; there may be evidence. I cannot say for rats, but certainly we have a great many non-domesticated mice and voles.

Kallmann: But the principle would be a negative selective factor, wouldn't it? Rate of reproductivity is somehow related to length of life.

Comfort: In terms of reproduction the effect of postponing senescence is surprisingly small when you have an animal which has this enormous random mortality. That was one of the points I was making from the evolutionary point of view, that you score much more by producing a lot of offspring young. The evolutionary score of high early reproductivity is going to be a good deal higher than that of potential longevity.

Best: Dr. Comfort, you seemed to use the terms "vigour" and "life" interchangeably at times.

Comfort: As regards "vigour", I have used the term rather than "longevity" and have used it, not in a strictly Darwinian sense, to mean the whole group of characters which tend to enable an animal to stand up to its environment. All animals either die from a cause that could kill them at any age, and which is not really germane to ageing, or from a cause which would not have killed them when they were more vigorous. You can probably defend the interchangeable use of "longevity" and "vigour" to that extent, unless you consider that "vigour" should only be used to mean vigour in the sense of characters which are strictly associated with Darwinian fitness.

Best: We need superb clinicians for tadpoles and other lower organisms. It is, of course, extremely difficult to decide even in human patients whether or not premature ageing is complicated due to infections or other definitely pathological processes.

THE STUDY OF THE AGEING OF CELLS

J. F. Danielli

Department of Zoology, King's College, London

That the senescence of mammals is in any way due to the ageing of cells is an assumption. The phenomena responsible for senescence as we know it may all occur at the supercellular level, and the differences which are observed between the cells of healthy adults and senescent adults may be secondary. However, there is no doubt that cells do senesce in themselves, and that even if senescence as we see it in mammals is not primarily due to senescence at the cellular level, nevertheless when other causes of senescence have been counteracted there will remain senescence of purely cellular origin.

In view of this position the essential aim of the study of the ageing of cells must be to detect the primary changes responsible for the senescence of cells, and then to devise methods for detecting such changes in somatic cells.

The cells to be studied

The first point which I wish to emphasize is that if one is to study senescence in cells, one must have cells which really are old. In some peculiar way this necessity has often escaped notice, and we find it said that studies on tissue cultures and on protozoan or bacterial cultures have shown that cells are potentially immortal. For the most part this is based on a fallacy. To begin with, if cells are continually growing and dividing, as they usually are in the experiments referred to, then they are not in any case old cells. For example, if the cells divide regularly at intervals of two days, then immediately after a division at worst half of the cell is two days old, one-quarter is four days old, one-eighth is six days old, and so on. The cell only becomes really old if its multiplication is

prevented. Secondly, cultures of proliferating organisms are more likely to conceal than to reveal any deleterious changes which may occur in individual cells. This is best understood by considering Fig. 1. The solid line represents the rate of normal proliferation of a culture, and the line B the rate of proliferation of a cell which suffers a deleterious change, at

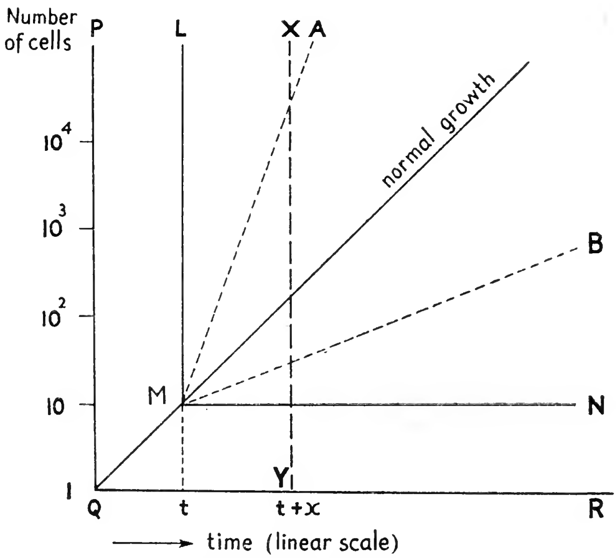


FIG. 1. Diagram to illustrate the changes in the proportions of normal and modified cells, A for a change favouring proliferation, and B for a change antagonistic to proliferation. PQR are the axes for the normal cells, and LMN the axes for a cell which becomes modified at the arbitrary time, t.

an arbitrary time t. PQR are the axes for the normal cells in the culture, and LMN for the modified cell and its products. The diagram illustrates the point that as time increases the proportion of modified cells in the culture steadily falls off. As illustrated, it begins at 10 per cent: after an interval (t + x) it will be 5 per cent, after (t + 2x) only 2·5 per cent, etc. Thus under conditions of steady growth any cell suffering a deleterious change tends to become insignificant: it is just

these cells which we should expect to be important from the point of view of the study of ageing.

We may also note that if a change takes place favouring proliferation (curve A), then the new cell line will outpace the original stock and ultimately exclude it if serial subculturing is used. This corresponds to formation of a tumour.

Finally, we should recall that few if any cells in a higher animal are undergoing continuous proliferation. In many tissues cell division may occur seldom or never, so that deleterious changes can accumulate. In other tissues, although cell division is common, it is based upon the division of stem-line cells which are retained: in these circumstances deleterious changes will accumulate in the stem-line cells.

Thus we see that, whereas in a continuously proliferating culture deleterious changes tend to be eliminated, in the cells of higher animals they tend to accumulate. Consequently our studies should be based upon cells which either are not proliferating, or which are stem-line cells.

Functions to be studied

Although qualitative differences between cells serve as a valuable guide to what should be studied quantitatively, qualitative studies are usually too subjective to be of great value in themselves. It is desirable to concentrate upon those cellular functions which can be measured. We may include:

(1) ability to grow and divide under standard conditions;
(2) ability to synthesize normal cell products, such as enzymes, hormones, and connective tissue fibres;
(3) ability to respond to stimulation, e.g. by hormones such as adrenaline and testosterone;
(4) ability to resist abnormal conditions, such as anoxia, extremes of temperature, malnutrition, bacterial toxins and toxic drugs.

At King's College we have recently begun such an investigation using *Amoeba proteus*. Studying point (1), Miss A.

Muggleton has found that if cells are kept for some months under maintenance conditions, without growth, they show marked differences from cells which are allowed to proliferate. Cells which proliferate normally follow the pattern shown in

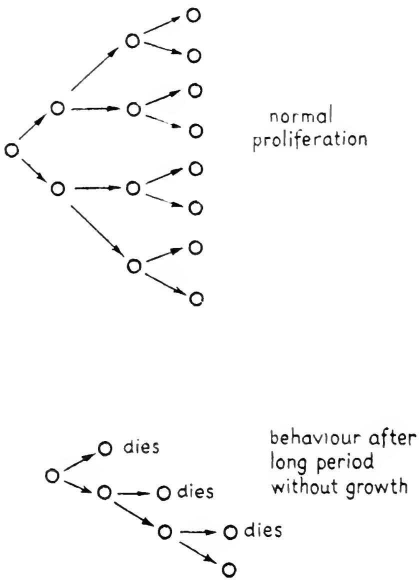


FIG. 2. To illustrate the effect of a period without growth on proliferation of amoebae. In the upper diagram, three divisions have given rise to eight identical cells. After six months without growth, restoration to growing conditions results in the products shown in the lower diagram, i.e. three cells are produced which die, and one cell persists, equivalent to a stem cell.

the upper part of Fig. 2, division being equational and all the daughter cells apparently equivalent. But after six months without growth, on restoration to normal growing conditions the result shown in the lower diagram is obtained—the daughter cells are not equivalent and one of the products of each division dies after a relatively brief life.

The primary nature of deleterious changes

When a change in cell behaviour with age is observed such as the change in daughter cell fate noted above, it is desirable to trace the change to a primary source. Among the factors to be examined are:

(*a*) mutations in chromosome major genes, polygenes, plasmagenes and homeostats;
(*b*) infection with viruses, etc.;
(*c*) accumulation of toxic products of metabolism.

Unfortunately most of the techniques which are necessary for examining these factors in somatic cells remain to be worked out. We may note that we do not know whether mutation of a single major gene in a tissue cell will result in cell death. It may be that the resultant deficiency can be made good by neighbouring cells. But the accumulation of many such mutations in the cells of an organ must limit the overall resilience and competence of the organ. Those cellular characteristics which are controlled by polygenes, i.e. inheritance of which is quantitatively continuous and not controlled stepwise by one or two major genes, would appear to be particularly likely to be involved in senescence, since mutation of several genes in a polygene set would not result in death of the cell, but in a quantitative diminution of the efficiency with which the appropriate cell activity can be performed.

Since the differences between somatic cell lineages appear to reside in their cytoplasms, we may also expect reduction in cellular competence to be caused by mutations of plasmagenes and homeostats.* The distinction between a deleterious nuclear change and a deleterious cytoplasmic change can probably be established by nuclear transfer, but the more detailed analysis of primary changes needs much more attention.

* A homeostat = an organization of macromolecules or of processes which is self-reproducing, and which normally carries out or controls at least one cellular function, e.g. chloroplasts, kinetosomes (Danielli, 1956. *Nature, Lond.*, **178**, 214).

The causes of primary changes

Where the primary change is an infection, or an accumulation of toxic products of metabolism, the cause may need little further elucidation. But where the primary change is in one of the cellular control mechanisms the establishment of the nature of the primary defect may throw little light on the causal agent. Where mutation has occurred it may be due to radiations, to mutagenic chemicals or to faulty replication. Changes in homeostats—resulting for example in a change in antigenic type—may be the result of an endogenous process, or result from exogenous agents such as infections, exposure to foreign macromolecules, or to promoting agents. So far as the study of senescence is concerned we are still almost completely in the stage of establishing changes in function with age, so that the establishment of the nature of primary causal agents is at present almost an academic point. Insofar as we are successful in carrying through the programme outlined, it will become less academic.

Conclusion

The final step in the study of cellular ageing will be to find means either of preventing or reversing the primary changes. One point which must be emphasized is that, even when all other sources of ageing have been removed, somatic mutation will remain. Thus if there is ever to be a complete control of senescence we shall need to be able to prevent, or to reverse, somatic mutation.

DISCUSSION

Baló: Our data on pathology in ageing cells are comparatively few, and one of these is the accumulation in ageing cells of some pigment substances. This is particularly well known in nerve cells; we find that in old age, intervertebral ganglionic cells, especially, are full of pigment granules, both in animals and in man.

Prof. Danielli spoke of changes in the function of the cells. I should like to contribute that in ageing cells one can find very marked changes in hormone or enzyme production. I have studied the pancreas of young and old animals and even of humans and found that enzyme production

decreases with age. Recently, Dr. Banga and I found that elastase production ceases in old age, so that in old age possibly there is no elastase in the pancreas. Prof. Danielli, have you had any similar findings in ageing cells in protozoa or in lower animals?

Danielli: It is quite true there are phenomena, in protozoa for example, which might be regarded as analogous, but I think the more important thing is that strictly speaking your observations are not an examination of cells in themselves but an observation on cells in a complicated organism, taken out and studied at different times during the life cycle. Now, that means that what you have seen may be due to a direct ageing of the cells, or may be due to some ageing process at a much more complicated level. My communication dealt exclusively with what would go on within a cell as a result of its own constitution and inevitable interactions with the inorganic environmental factors, and not things which might occur as a result of relationships between cells. So I would think that, whereas looked at from the point of view of whole-animal studies, those are very interesting investigations which you have made, yet looked at from the point of view of cellular studies they are not so interesting because one does not really know what it is that is affecting the cell, whether it is something which is intrinsic to the cell itself, or whether it is something that is intrinsic to an organ or to a whole animal. My reason for limiting very sharply my communication to the cellular level is that I think that one of the things we have got to do, in the study of ageing, is separate out those things which are fundamental to the extracellular level from those which are fundamental to the multicellular level.

Friedman: Prof. Danielli, I respect heartily the manner in which you have circumscribed the limits of the field that you have been discussing, but I wonder, when dealing with the problem of ageing and the ageing of cells, whether we really can so sharply isolate the cell from its environment? Can we, for instance, speak of the ageing of the cell without speaking of the ageing of its environment? If one studies, as we have done, sodium transport systems in smooth muscle *in vitro*, one can get entirely different results depending on the medium one uses and, indeed, on whether one uses fresh plasma or old plasma or fresh serum or old serum. Now, there the environment has, in a sense, aged; can one separate off the ageing of the environment from the ageing of the cell even though I admit that, ideally speaking, one would like to?

Danielli: I think one has got to if one is going to understand the details of any process of ageing. To begin with, in any separate cell the overall genetic constitution may be such that that cell has a limited life irrespective of anything that may be environmental. Secondly, it is quite inevitable that there will be mutagenic factors acting upon that cell—radiations, mutagenic chemicals, and so forth—which are in only a very special sense an environmental factor and which set an ultimate life to that cell; obviously, somatic mutation will ultimately cause every cell to die which does not first die from other causes. We have to separate out these factors and assess them separately from those things which come in as a result of interactions with the environment. It is not that

I think that interactions with the environment are of negligible importance, but that we should separate these things quite clearly from one another.

Best: I can see, Prof. Danielli, that you were driven to the experiment of preventing your unicellular organism from dividing. But you had to do something different to accomplish that, you had to make a change. Can you do this in a variety of ways and get the same result every time?

Danielli: The experiment I have described to you was done simply by restricting the amount of food that was provided. I cannot say whether it can be done in other ways, though later I may be able to do that.

Tunbridge: I understand you have taken cells of different ages and transferred the nucleus, putting it into cytoplasm of a different age; to what extent has such work enabled you to determine the duration of life of the cytoplasm of the cell?

Danielli: We have not done any work yet on nuclear transplantation in this particular connection. The experiments I described to you were made to define the field where such operations were possible.

Olbrich: Does the fluid content diminish in these ageing cells of yours?

Danielli: No, there is no characteristic change in that, as far as our measurements have gone, but we have not made accurate assessments of dry weights of single cells.

Best: I don't quite follow that one-half of a dividing cell is old and one-half new.

Danielli: Of the macromolecules which are present in the cell just before it divides, at least half (since it is doubled in dry weight) will be new macromolecules.

Best: But these will be divided between the two cells?

Danielli: Perhaps you could even, in some cases, take a single macromolecule and say half of it is new and half of it is old. But since the amount of living matter has doubled and it is doubled in a finite length of time, say two days, the life of at least half of that new cell has been only two days.

Comfort: Have you any information about those ciliates, and other such organisms, in which you can identify which half of the dividing cell gets the old macromolecule and which half gets the new? I understand that in some cases you can see which is the daughter cell which gets the new organelle and which is the mother cell which gets the old one.

Danielli: French protozoologists, e.g. Lwoff, have done fascinating work on that. As far as I recall, the usual thing that happens with kinetosomes is that the daughter cells will both get some old kinetosomes, but then as the new organism grows after cleavage, each of these will multiply in some way, so that after division has taken place repeatedly you have no cells which contain 100 per cent old kinetosomes.

Comfort: In an organism like *Stentor*, you could make a form of life table.

Danielli: Yes, that could be done by isotopic labelling and would be an interesting experiment.

Landowne: To what extent could the cell that did not divide be considered to be old because of the variable percentage of replaceable

material? Are you not just shifting the focus of observation from a multi-individual or a multicellular community to the multimolecular community of the single cell, and does this really escape any of the problems?

Danielli: This was one of the problems I was hoping not to have to deal with extensively! Here, one has to move into a little more detail about the relationships of genes and macromolecules. As far as I understand the situation at the moment, the function of the nuclear genes is to control the manufacture of particular types of macromolecules: I would hesitate to say that the macromolecules which are made under the control of one gene are always absolutely identical. The things which a particular gene "makes", generally speaking, are presumably very similar, but I should hesitate to say that there is any known way of establishing that they are absolutely identical. Secondly, there is the process organizing these different types of macromolecules into particular functional units, which again is genetically controlled but which is a totally different process and different level of activity of the cell compared with the actual manufacture of the macromolecules. I can see that in senescence some degree of breakdown may take place both in the manufacture of the macromolecules and in competence to organize them into specific structures, and I think each of these things must be examined separately.

Landowne: Certainly the macromolecules, once made, are relatively stable in their environment. Do you consider there is much physico-chemical repair and reversibility in position and in bonding within a constructive bit of architecture, so to speak?

Danielli: That depends on which sort of molecule you look at. If you look at deoxypentose nucleic acid in the non-dividing cell, the evidence is that it does not change much. If you look at haemoglobin, you will not find it greatly changed, with the exception, perhaps, of the haem moiety. If you look at some adaptive enzymes you will find that they don't seem to change very much. On the other hand, Gale's work shows that amino acids can pop in and out of some sort of proteins quite easily under some circumstances; and if amino acids keep on popping in and out of a molecule, how can you call it either an old molecule or a new molecule?

Landowne: If they continue to pop in and out with the same rapidity and in the same manner over a period of time, then the molecule is behaving as a young molecule. When the exchange is altered, whether it is being altered intrinsically or extrinsically, we have a change, and this might give rise to something which is called an effect of age.

Danielli: That then would be a failure in the control process which determines what pops in and out.

Landowne: Yes, and is this not exactly the sort of thing which we deal with in cells that divide?

Bourne: I think the same problem arises in consideration of the skeleton. There is a constant turnover of calcium and phosphorus in the skeleton and after a time one has a completely different series of molecules composing one's skeleton to what one had earlier. Are you

dealing then with an older skeleton when, in fact, you have got one with completely new molecules?

Danielli: I don't regard the composition, shall we say the newness, of any particular atom in a particular macromolecule as being important; what I do regard as being important are the processes which control which atoms shall be present in which places, and it is at these control processes that you have to look for some of the fundamental changes which occur in ageing.

Gillman: I realize that my remarks that follow are not on the cellular level; nevertheless, there is some evidence that the view which Schoenheimer and his collaborators put forward concerning the speed of interchange of molecules in certain tissues (such as bone, mentioned by Dr. Bourne) does not seem to hold to the same extent in certain other apparently important connective tissues. According to recent studies, the rate of turnover of molecules in structures like collagen is relatively slow. In fact, I was rather surprised to learn that it is believed that collagen fibres, once laid down, e.g. in a more or less mature rat, would stay in the animal to the end of its life. However, Dr. J. Gross has recently indicated that even an extract of collagen fibres actually ages, even at about 2° or 3° C—ageing here being judged by the solubility and other characters of the extract. I don't know how far decrease in speed of turnover is related to ageing, or, as Prof. Danielli has mentioned, how far ageing affects the kind of molecules which move in and out of a particular organized structure. I mention this possibility because there are certain kinds of structural proteins within cells and even within extracellular fibres which may perhaps decrease their speed of molecular turnover with ageing more than others.

Danielli: Yes, that is absolutely certain; deoxypentose nucleic acid scarcely turns over at all and it is, in a sense, one of the most important control elements.

Gillman: Are there any others that you know of?

Danielli: American workers on adaptive enzymes have been able to show that in some circumstances the turnover of an adaptive enzyme is extremely small compared with the rate of synthesis of adaptive enzyme in the presence of the activating substrate. But Schoenheimer's experiments make it quite clear that many other proteins are subject to rapid turnover.

Gillman: Prof. Danielli, you made a remark a moment ago which I would like to question if only to get clarity on this point. You said that certain structures in the cell were the more stable in that they did not change so rapidly in the cell, and you apparently went further and implied that the stability of a molecule within a cell gave that molecule a particular regulating function within the cell.

Danielli: No, I did not wish to convey that at all. One thing that I did want to emphasize, however, is that the fact that deoxypentose nucleic acid does not interchange its parts very often does very likely give it a special stability which it would not otherwise possess. Every time a nucleotide or an amino acid is moved in and out of a macromolecule, a possibility of error comes in, when carrying out the synthesis. So that

as long as you are not doing these things to a molecule the possibilities of change are diminished, and consequently the permanence of that particular feature is more assured.

Gillman: Therefore, if you are going to discuss any implication for the survival of the cell in that particular case, the less the molecular chains alter and, therefore, the less the chance of error, the greater the survival rate. Is this the line of thinking?

Danielli: That is a generalization I should prefer not to make until I had some experiments which justified it. I should say this idea was one of those stones that you lift up to see what kind of animals are lurking underneath!

Gillman: The line along which our group is thinking is that change and maintained capacity for change is the essence of youth far more than stability. This is characterized in many features of growing organisms and there seems to me to have been too much of a tendency in much of the modern literature to regard change as a bad thing in terms of maintaining juvenility or youth.

Best: The interchange of fatty acids which Schoenheimer first showed between, let us say, liver and muscle tissues, might be of great importance in this connection. There are factors which vary the rate of this "flux". They have not been worked out carefully, but it would be extremely interesting to explore the effect of ageing on the rate of this interchange.

Gillman: We have indications that the slowing up or even stoppage of molecular change, in certain structures in connective tissues, is more closely associated with ageing and degeneration of those tissues than is the rapid turnover seemingly characteristic of the young.

Danielli: I do not think anyone knows just why it is that certain of the proteins are apparently in a very dynamic state so that the amino acids are popping in and out, and other intracellular proteins are in an extremely stable state. I don't think we know the answer to that. But I think a fundamental point, so far as cells are concerned, is that the feature which distinguishes a living system from a dead one is that you have continuous change which is operating within fixed reference points, so that the whole system is controlled through changing. What happens to any particular molecule is unimportant unless that molecule is a key one in the control processes, such as deoxypentose nucleic acid. If a controlling molecule like DNA is exposed to rapid metabolic changes then the possibility of errors creeping into its constitution are much greater than if it is maintained stable. On the other hand, you cannot have everything in a cell stable or it would cease to be living—it would be a fixed cell. In fact, the whole character of a living cell depends upon its occupying a "halfway house" between complete plasticity and complete stability. Take for instance water, in which none of the atoms are held in a specific position for any perceptible length of time; you could not base a living organism on that sort of structure. Equally, if you take for instance a metal, the atoms may occupy one position for a great length of time. Again, you cannot base a living organism on that. The characteristic property of living cells is that they are made from

macromolecules which are very often stable in one dimension, along a chain. You have covalent bonds in the chain which give great stability. And yet the relationships between adjacent chains are based upon hydrogen bonding, and have almost the same lability as water; so you have this curious compromise between absolute rigidity and great lability. I think the extraordinary plasticity of living cells depends on this sort of thing.

Bourne: Does this plasticity, which I suppose is represented by the rate of flow in and out of molecules and compounds, vary between old and young cells?

Danielli: I do not know.

Bourne: In a sense the processes that are going on in the cell are comparable with an eddy in a molecular whirlpool, and it is only the pattern of the eddy which is constant, with perhaps the pentose nucleic acids sticking out like rocks in a rapids.

EXAMPLES OF REACTIONS TO STANDARD STIMULI AT DIFFERENT AGES

K. J. Franklin

Department of Physiology, Nuffield Research Unit, St. Bartholomew's Hospital Medical College, London

Special research on ageing began in this Department only fifteen months ago and it has been limited to mammals bred (with extremely few exceptions) in the Department, and to Man. The starting point was the idea that reactions to definite stimuli at different ages could be measured, and the changes in reaction with the passage of time could be determined. Results could thereafter be analysed, and when sufficient researches had been made, one might begin to get a better appreciation of the meaning and tendency of ageing. This directive was not to be the sole one, but it has proved to be the major one, in the Unit's work to date.

The first study, by Carter (1956), was of the response of the gingival epithelium of the Merion rat, *Meriones libycus*, to brushing; it was intended as a precursor to the studies on human subjects which are mentioned below. The brushes used for the rats were specially made. Male subjects in three age groups were tested, the gum on one side of the upper jaw being brushed for one minute, twice daily, for four weeks, while the other side served as a control. The brushing led to a significant increase in the thickness of the cornified layer, and the height of the epithelial papillae was also increased (Figs. 1 and 2). Within the range studied, however, which was from early adult to middle age, ageing *per se* was without significant influence upon the thickness of the cornified layer or upon that of the epithelium. A supplementary study of animals between birth and early adult age has therefore been begun, but results are not yet available.

The Unit's second research study was upon the renal blood content, and its variations with age, in the rabbit; in this study, Lindop (1956) made one of the earlier uses of radioactive isotopes in studies of ageing, summarized as follows: 50 rabbits of a standard strain, aged from 1 day to 3 years, were found to have renal blood contents (ml./100 g. renal tissue) ranging from 50 neonatal to 41 between weaning and puberty, and thereafter to 30 in adult life. These levels are the resultants of all stimuli acting together under the conditions

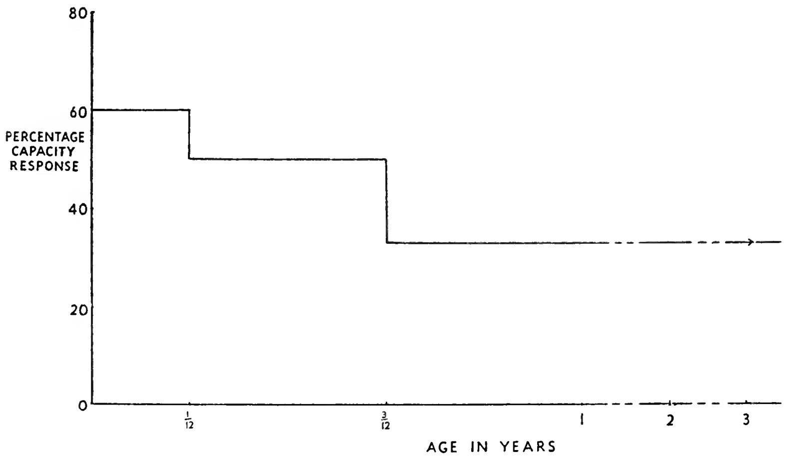


FIG. 3. Diagram showing percentage capacity to reduce renal blood content at different ages. Subjects: 50 Rahere rabbits.

obtaining. The standard extra stimulus in each case was a period of 20 sec. asphyxia of the whole animal (Franklin, McGee and Ullmann, 1951), such asphyxia being permitted to act reflexly upon one kidney only. It caused a drop in renal blood content to 20 ± 4, irrespective of age, so the actual drop decreased with age from neonatal to adult (Fig. 3).

The third research, also by Lindop, is a study of thyroid function, and in it she has once again used radioisotopes for the physiological, non-structural assessment of function. Tracer doses of radioiodine have been used in rats to study

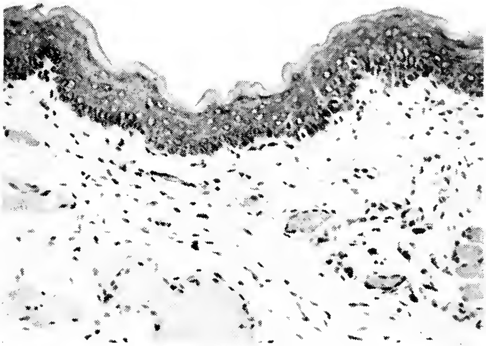


FIG. 1. Unbrushed masticatory mucosa of Merion rat No. A 3. Scale in μ.
Reproduced by permission of *Brit. dent. J.*

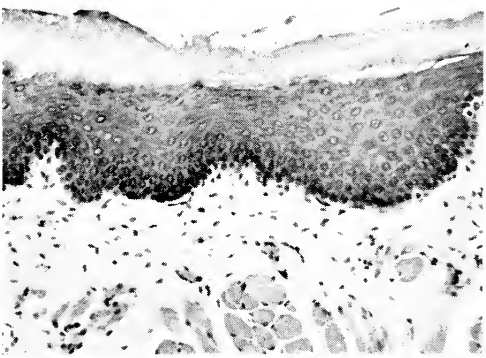
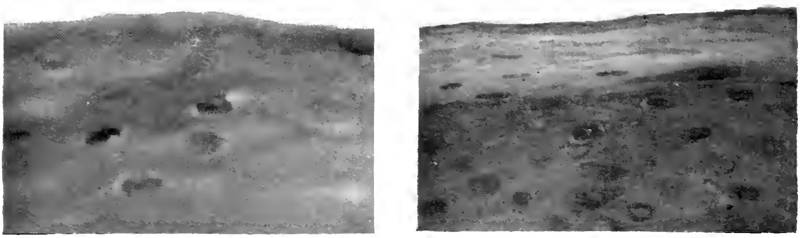


FIG. 2. Masticatory mucosa from opposite maxilla of same rat after 1 month's brushing. Scale in μ.
Reproduced by permission of *Brit. dent. J.*

[*facing page* 52



(a) $40\ \mu$ (b)

Fig. 6. Human case No. 37. (a) Before treatment C. L. nil: (b) after treatment for one month, $20\ \mu$.

one activity of the thyroid, namely, iodine uptake, as a function of age. This method has the advantages that it is independent of the histological picture of the gland, and that the test itself does not disturb the subject's physiological condition. The disadvantage is that to get an absolute measure of uptake the animals must be sacrificed. The method has, however, been based on measurements in 371 rats, aged from

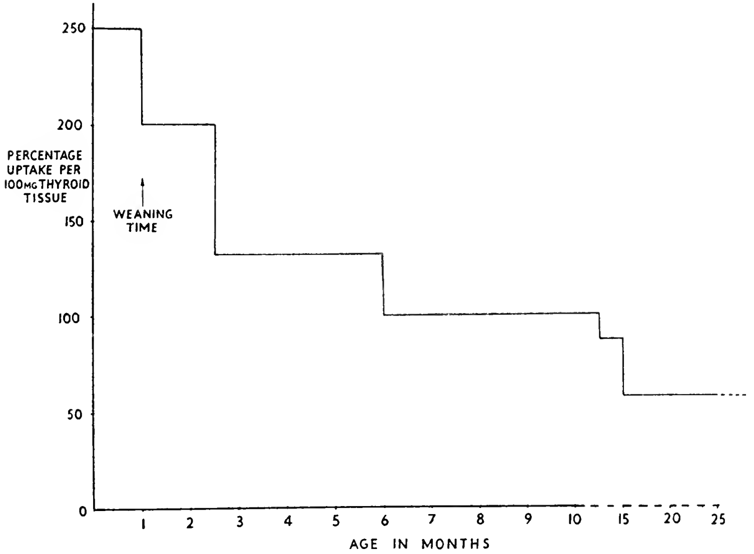


FIG. 4. Graph showing 24-hour percentage uptake of tracer dose of radioiodine per 100 mg. thyroid tissue. Subjects: 371 Wistar rats.

1 day to over 2 to 3 years old, and with the normal percentage uptake established this value can be used as a standard for survival experiments in which surface methods of counting, as with a collimated Geiger counter, can be used. The series falls into two groups, as the percentage uptake of radioiodine is directly proportional to the iodine intake in the diet. Thus, the first group are the foetal and neonatal animals, fed on milk only, and the second group the weaned animals, fed on a diet of fixed iodine content.

The results themselves show the immense activity of the thyroid gland in the neonatal period, compared with its activity in the adult and aged stages. The weight of the thyroid tissue varied from 1 mg. in the neonatal animals to 25 mg. in the adults. The average uptake was 20–22 per cent in the latter, and *ca.* 10–12 per cent in the former. However, expressed as a function per 100 mg. thyroid tissue (Fig. 4), the younger gland shows the higher activity and thereafter there is a gradual fall-off with increase in age.

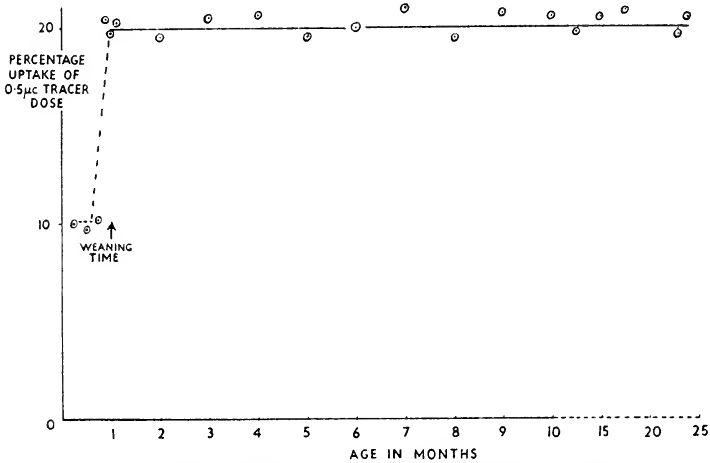


FIG. 5. Graph showing 24-hour percentage uptake of tracer dose of radioiodine by thyroid gland at different ages. Subjects: 371 Wistar rats.

Fig. 5 shows the relative constancy of the percentage uptake after the rats are one month old. This can be used to determine the relative sensitivity of the thyroid gland to internal irradiation at different ages. In an attempt to study foetal thyroid uptakes, full-term mother rats have been injected with a known dose of radioiodine, and the foetuses have been sacrificed after a further 24 hours, following either spontaneous birth or delivery by Caesarean section. They have been found to take up *ca.* 2·5 per cent of the dose given to the mother. Now that this level of uptake is established, it should be possible to

determine the effects of radioiodine, injected into the mother, upon the embryos and foetuses at different stages of development. This will be of interest in the light of Van Middlesworth's (1956) report on the surprisingly high values of radioiodine found at necropsy in the thyroid glands of cattle, sheep and human beings on account of fall-out from nuclear weapons. The long-term effects of this persistent source of internal radiation need to be studied in relation to development and longevity of the exposed lower animals and of Man.

The sensitivity of the rat thyroid to internal irradiation has been studied and an average dosage of 10 μc/100 g. body weight has been found sufficient to destroy thyroid activity in adult rats. The effects of this dosage, and of much larger and of much smaller ones, are being tested on the long-term thyroid activity. In a first series of 30 animals, following doses of 25 μc, no diminution of function has been found as measured by radioiodine uptakes after 35 days. This dose of 25 μc is equivalent to an irradiation dose of 20,000 r over this time, if the effective half-life is taken as 4 days in the adult rat. This amount of r is equivalent to a human therapeutic dose.

In summary, normal levels have been established to date for the percentage uptake by rats of a tracer dose of radioiodine at monthly intervals from birth to two years. This standard can now be used for further studies of thyroid function as affected by age, and of the effects of continuous low-level irradiation on ageing of the individual animal and on litter development.

The fourth research is the human counterpart of the gum-brushing study in Merion rats. It arose out of a talk with G. A. Cowan, Dental Surgeon to St. Bartholomew's Hospital, and its object was to test a belief, prevalent in many dental circles, that cases of chronic inflammation of the gums can often be relieved by the simple expedient of regularly brushing the gums with a stiff toothbrush. It seemed that a simple and standardizable test of this kind could also be used for a comparison of reactions at varying ages, and it was decided to proceed

along such lines if results of a simpler study (see above) on Merion rats proved promising. A very large programme was agreed upon by G. A. Cowan, his registrar, J. L. Marsden, this Department's Senior Lecturer in Histology, F. J. Aumonier, and the author. The subjects were to be patients of all possible ages and both sexes, and biopsy specimens were to be removed with agreement from all requiring extractions, the first biopsy being made coincident with first attendance at the Outpatient Department. The total number of such biopsies was to run into four figures so as to allow for proper statistical treatment of findings subject to many more variables (e.g. left- and right-handed brushing, varying loss of teeth) than is the case in Merion rats.

The scheme has been put into practice in the hope that it will give evidence of variation with age in the response to brushing, as well as be of direct dental relevance without reference to age. Patients are instructed to brush their gums twice daily using a standard dentifrice and brush, both of which are supplied free. The brushing ritual consists of twelve single strokes from gum to tooth on each of four "quadrants" into which each jaw is divided, giving a total of 96 strokes in each of the two daily brushings. Biopsies are taken one, two, and three months after the beginning of treatment. The biopsy material is fixed in Bouin's fluid, embedded in paraffin wax, sectioned perpendicular to the epithelial surface, and stained with haematoxylin and eosin.

So far 53 patients, aged from 6 to 67 years, have been studied. The majority fall into the teenage, the twenties and the thirties, other decades being as yet represented by very few individuals. It is still premature to try to relate changes in the response to the stimulus to changes in age, but such indication as exists to date is in favour of an increase, rather than the opposite, with increase in age. Perhaps this is a puberty effect, and that possibility will be borne in mind. The response to the set stimulus is a noticeable thickening of the cornified layer of the epithelium (Fig. 6*b*); the whole epithelial sheet also tends to thicken but, as the

pattern of the dermal papillae is very variable and changes profoundly in places less than 1 mm. apart, this is a far less suitable index of response than the behaviour of the cornified layer.

It is tempting to speculate whether the power to shed intracellular water may be the underlying property, and this property be increased by age. So far the only cases which have shown anomalous changes in the thickness of the cornified layer have been females. As no records of the menstrual history of female patients have been taken, the possibility of endocrine influence upon epithelial responses must be borne in mind.

In two of the four researches mentioned above we have tried—and are still, in fact, trying—the reaction of rat and human gums to the extra stimulus of being brushed with tooth-brushes. Unfortunately, in the case of the rats, there is no significant variation in the response from the time of puberty onwards, and it is not easy to devise a fully comparable stimulus for use in rats during the neonatal-prepubertal stages; the gum area is small enough even in the older animals, and there are other problems. In the human subjects a trouble not experienced in the rat series is the difficulty of getting comparable numbers of biopsy specimens in all age groups, and above I have referred to other difficulties. I think we shall eventually get an answer in respect of human ageing which will be more declarative than that from the rats, and we have already had a minor indication of some factors that may be concerned in that answer. The final comment that I will make on these gum experiments is that measurements dependent upon fairly complex histological procedures are not entirely satisfactory and are certainly laborious and time-consuming. One comforting point about the human research is that the brushing has already demonstrated therapeutic powers so that, in addition to any contribution the work may make to the study of ageing, it may also make one to the furtherance of dental hygiene and thereby to a longer and healthier lifespan for vast numbers of people.

The kidney research tested the change with ageing in a particular capacity of the organ, viz. its ability to reduce its blood content in response to the reflex stimulus produced by a given length of exposure of the whole animal to asphyxia, and it showed a marked falling-off in this capacity from birth to adult life. The technique used in measuring is of interest for its modernity, and the findings should be of value in any final explanation of the kidney's behaviour as a lender of blood to other parts in response not only to asphyxia but also to many other stimuli.

The thyroid research, testing one function of the gland, namely, iodine uptake, gave an answer even more emphatic than the kidney research in respect of a falling-off with age. The technique was again modern, making for greater speed in obtaining more acceptable results.

I think that the reactions to some other stimuli may prove to be increases in functioning up to puberty, with no marked changes thereafter; intra-uterine gonadotrophin effects can on occasion complicate such simpler pictures. I can also imagine a third class of results not to be described in terms of variations in reactivity to standard stimuli, but that is going outside my terms of reference.

When a sufficient number of findings have been made about ageing in studies of reactions to standard stimuli at different ages, it should become possible, through this and other information, to produce a properly chronological account of the physiology of ageing which will be a whole-life story, written in an appropriate terminology and infinitely more exciting than the best accounts at present available. This is in no way a belittling of such accounts, but merely the *credo* of one person in respect of the future of our as yet very young Science.

Acknowledgement

I wish to acknowledge the co-operation of my various colleagues which has made this paper possible, the fine assistance our Departmental and Animal Technicians have given us, and the liberal financial support afforded by the Trustees of the Nuffield Foundation.

REFERENCES

CARTER, S. B. (1956). *Brit. dent. J.*, **101**, 76.
FRANKLIN, K. J., McGEE, L. E., and ULLMANN, E. A. (1951). *J. Physiol.*, **112**, 43.
LINDOP, P. J. (1956). *J. Physiol.*, **132**, 13P.
MIDDLESWORTH, L. VAN (1956). *Science*, **123**, 982.

[Discussion of this paper was postponed until after the paper by Prof. Verzár.—EDS.]

STUDIES ON ADAPTATION AS A METHOD OF GERONTOLOGICAL RESEARCH

F. VERZÁR

Physiological Institute, University of Basle

SINCE the present conference is to deal with "methods" of gerontological research, I wish first to explain why we have chosen for our method of attack the study of changes in adaptation which occur with increasing age throughout the individual's life.

The word "adaptation" has been much misused in recent years, as if it depended upon the special activity of one endocrine gland only. It cannot be emphasized strongly enough that such a concept is a misunderstanding of the fact that "adaptation is the general capacity of living organisms to live under continuously changing conditions". In fact, the adaptation capacity of fluent metabolic equilibria is probably the best characterization which can be given for "life". When this adaptability decreases, ageing begins.

This viewpoint may bring us into conflict with the idea that ageing starts with conception or, as others like to say, with birth. No doubt there are retrograde cell processes even in the embryo, but they are changes in the ontogenetic evolution, which are not a decrease in adaptability. On the contrary, evolution means increasing adaptation to changes in conditions of life. When the human embryo loses its gills and tail, it has recapitulated a part of its adaptation from former generations to a completely changed evolutionary period.

Birth is in fact the greatest adaptation process, when foetal respiration, circulation and nutrition have suddenly, in minutes or hours, to be transformed to meet completely new life conditions. Thus, even if we see that the placenta and the umbilical cord show histological degenerative changes,

which may somehow remind us of what happens in later life in degenerating cells, this is not true ageing: on the contrary, these are processes of adaptation.

When the newly born infant adapts itself to the completely changed conditions of extra-uterine life, it acquires the capacity of heat-regulation, of enteric nutrition, visual and acoustic sensitivity and motor activities of increasing complexity. Thus, it increases the range of its environment and acquires more and more potentials to meet the constantly changing conditions of life. Its evolution in the first years of life is principally an increasing capacity for adaptation.

The evolution of the individual's nervous system finally provides it with the capacity to live in the Arctic and in the Tropics, at temperatures between $-40°$ and $50°$ C, to live at pressures between 150 mm. Hg and 5 atmospheres, to withstand dangerous radiations and to meet the psychical challenges of the business world or of the political world; in other words, it enables the growing individual to learn to adapt himself to more and more difficult circumstances.

These processes of adaptation produce a maximal capacity and effectiveness in many or all the necessary organs. To enable the individual to survive, his endocrine structure must be normal, i.e. healthy, because endocrine hormones influence the metabolic activities of muscles, of liver, of kidney and of so many other cells. A lack of insulin will be as deleterious as a lack of corticosteroids. A disharmony between the function of all organs will be disastrous. If his kidneys cannot stand periods of thirst, it will be as destructive for his life as if his brain neglects to watch for his deadly enemy. Training of all organs to almost—but not complete—maximal capacity goes on throughout the years of extra-uterine life. This is adaptation.

In the young animal, adaptability overshoots its average requirement to a point where large reserves of adaptation are accumulated. Young animals "play" with the mother to increase their forces for any possible emergencies. The minute volume of the heart, the circulation blood volume, the

minute volume of respiration are never used to maximal capacity during normal life; but adaptation produces a margin of safety for all eventualities.

However, from a certain age on, adaptation as a whole becomes less complete. More and more, a decrease of adaptation capacity reveals itself. This is completely different from what happens in earlier life. When the 15-year-old boy shows a decreased accommodation capacity of his eyes, and the near-point of his eyes is no longer 12 but perhaps 20 cm., then something has started which does not increase his adaptation to the variable conditions of life. At the same time, he is already losing the capacity to hear 20,000 Hertz waves. Somewhat later, his athletic powers decrease, being based on the flexibility of his joints as well as on the capacity of his lungs and heart to provide for maximal effort. Nobody will expect the 22-year-old Olympic winner to be in the same condition at the age of 28 or of 30 years. The adaptation to sight, to hearing, to muscular hyperfunction, has decreased.

In psychical capacities it is more difficult to see the early start of the decrease in adaptation. It is, however, well known that the training of animals is easier with young individuals. Man does not stop learning, at least if he embarks on a scientific career, but he certainly loses the capacity to learn very quickly by heart. Even this fact may have different explanations. The limiting factor in the taking-up of new knowledge may be, not the capacity to remember, but rather the accumulated quantity of continuously remembered facts. From the point of view of psychical ageing in humans, as well as in the old leaders of a herd of animals, the factor of experience brings in a rather aberrant evaluation of the old individual. While he is less efficient in the sensory and muscular capacity, he may be increasingly valuable in a certain integrating psychic capacity, owing to his greater accumulation of experience in many different situations.

When the capacity to accommodate visually has reached its end, the human individual has reached his fiftieth year.

In conditions of primitive life, he will now still be able to recognize his neighbour in the cave, but he will not notice until too late his enemies in the jungle. This is the same age at which, in his female partner, ovulation ceases, and thus both become superfluous for the upkeep of the race. From the point of view of the race, they are aged. If we did not respect in man the mental part of his life, we could rightly say that his lifespan should end at the fiftieth year. It is only the mental powers which have a continuing value with the prolongation of his lifespan, and I think this provides an answer to the question of ambition to prolong life. Individual mental experience, and the capacity to transfer this experience, is the only advantage of the prolongation of a man's lifetime, from the point of view of the race. A specific human factor seems to enter gerontology at this point.

Thus, a decrease in the capacity for adaptation is the main characteristic of ageing. If we try to understand ageing, we should try to understand those changes which make adaptation less possible. It would be naive to see the cause of decreased adaptation only in apparent morphological changes. It is true that these exist: for instance, the athletic capacity of the young individual alters if his tendons and joints become rigid. In this case, apparently macro- and microscopical tissue changes seem to explain the decreased capacity, and the problem is: "What causes the morphogenic changes?" But adaptation is a dynamic process. The capacities to integrate complex functions diminish, as shown by the following examples:

The capacity of heat regulation decreases with age (Hügin and Verzár, 1956*a*). This needs a central integration between chemical and physical processes, i.e. heat production in the muscle and heat release by conduction, radiation and evaporation through a regulation of blood circulation and also evaporation of water from the lung and skin.

Both processes are decreased in the aged. Old rats are unable to protect themselves against temperature decrease in a cold environment, and they are also less able to

protect themselves against the increase in body temperature in a hot environment. It is true that both cases need the co-operation of many organs—endocrines, muscles, circulation—but the main trouble seems to lie in a decreased central capacity of regulation, probably in the central nervous system.

This is even more obvious in the next case, the loss of adaptation to low oxygen pressure (Verzár and Flückiger, 1955). At 0·5 atm., body temperature drops several degrees but is restored to normal in one or two days. Old animals, however, lose the capacity to restore this drop in temperature. Furthermore, they also lose the capacity of "retained adaptation". This means that in young animals the heat regulatory centre or the body cells in general can, so to speak, learn to live at a low oxygen pressure, but this is not possible in the old animals.

Certain other powers of adaptation still seem to be present in the aged animal to a remarkable degree, for instance, work adaptation by means of hypertrophy.

We have tested this in the heart (Hügin and Verzár, 1956*b*) and found that an increase in resistance and consequent high blood pressure leads to left ventricular hypertrophy in 5 days in the rat. In a decisive number of old animals, such a work hypertrophy of the heart muscle was equal to that in young animals. Also, the compensatory hypertrophy of the kidney, after extirpation of one kidney, was hardly less in old animals than in young ones (Verzár, 1955*a*). Similarly, the compensatory hypertrophy of the adrenals showed about the same values in young and old animals (Verzár, 1955*a*). However, we recognize the difficulties arising from the competition of atrophic processes which may run parallel to hypertrophic ones in these old animals.

Experimental psychological research shows that the ability of adaptation to learn a maze in order to get food (thus a process of learning) decreases measurably with age (Verzár-McDougall, 1955). Furthermore, the capacity to remember a learned task decreases in certain aged individuals.

It is, however, astonishing how these experimental psychological studies have revealed enormous individual differences in psychical ability in old animals of equal age, from a memory comparable to that of a youth up to complete senility with loss of memory.

The loss of memory seems to show a basic parallelism to our findings on retained physiological adaptation to low pressure. The old individual loses the capacity to readapt himself to a situation which he experienced some time ago, and to which at that time he had become adapted. If this parallelism is accepted, we may rightly ask whether one should search here for a basic cellular process—perhaps in the central nervous system only—which in young animals leads to "retained" reactions or to "remembrance", and which is damaged or is lacking in the old.

Such were our thoughts when we turned to the study of the physical adaptation capacity of connective tissue. Adaptation to mechanical stress needs an adaptability of collagen and of elastic fibres. Much has been said about the decrease in elasticity with age. In the arteries, this leads to decreased adaptation to the changes in blood pressure. The mechanisms of these changes have been explained as an effect of a diminution of elastic fibres and an increase in elastase activity, either directly or by a change in the concentration of an antagonist. Thus, changes in enzyme activity have been suspected as a cause (Banga, 1953).

A remarkable change in our attitude is in progress at present owing to the fact that it is understood that collagen can change into an elastin-like substance (Hall, Reed and Tunbridge, 1952, 1955*a* and *b*, 1956). I only wish to mention this much-discussed territory in order to show that the ageing of collagen has become of great general interest. We studied it with the hope of finding such basic changes as might help us to understand the decreased adaptability for mechanical purposes in the aged. We chose a collagen tendon fibre, and we found that with increasing age an interesting phenomenon starts. The older the animal, the larger the loads necessary

to inhibit the thermal contraction of the fibre. We have reached the conclusion that the explanation of this phenomenon must be an increase in "cross-linkages", perhaps of hydrogen bonds, between the collagen molecules of the tendon fibres in the aged (Verzár, 1955*b* and *c*, 1956). The same mechanical changes of thermal contraction as in the isolated collagen fibre can be seen in whole skin and also in the whole nerve (Verzár, 1955*b*).

It is possible that a mechanism of ageing of filamentous proteins, such as collagen, has thus an even more general interest. Many biologically important proteins are filamentous molecules, like insulin and the nucleotides of the chromosomes, etc. If ageing means an increase in "cross-linkages", then this process might occur also in other proteins in a manner similar to that seen in the tendon of the rat's tail.

If it were realized that an increase in cross-linking is a main process of ageing in molecular dimensions, then it might even be possible to approach experimentally the question of its decrease. Curiously enough, the tanning industry may already have the greatest experience, one which we can use in future studies on ageing (Björksten, 1951; Wood, 1954). A destruction of cross-linkages in general may be able to postpone ageing, especially in certain tissues, perhaps in the collagen-containing tissues of joints and tendons.

Parallel to these changes in the collagen fibre, there are others which break it down to elastin-like tissue (Banga, 1953; Hall, Reed and Tunbridge, 1952, 1955*a* and *b*, 1956). These changes are in many ways similar to those which can be shown experimentally by thermal and some chemical influences on the collagen fibre. The thermal contraction starts with a breakdown of mucopolysaccharide from the filamentous protein of the collagen fibre; if the temperature is high enough, this leads to a contraction. This process can be much accelerated, or the temperature at which contraction occurs can be much lowered, by acids. I only want to point out, in a rather hypothetical way, that we are approaching here the problems of the causes of elastosis of the human skin.

We are also moving nearer to the solution of another problem: the continuously increasing capacity of tissues to bind calcium (Freydberg and Verzár, 1956). The older the animal the larger is the calcium turnover in aorta, lung, brain and tendons, while at the same time it has rapidly decreased in the bones. It has been pointed out by others (Banga, 1953) that, with the breakdown of collagen, di-amino acids which have a great affinity for the calcium ion become free in the fibre protein. The calcification of the tissues may be a direct consequence of the same process which changes the collagen fibres into elastin-like tissue and also breaks down the elastic fibre into amorphous material.

With this we have reached the realm of protein chemistry as a fundamental part of research on adaptation changes, which might become the basic phenomenon of ageing.

It will, I believe, be the main object of future research on ageing to understand the facts which are responsible for those changes of the proteins of cells and tissues which lead to the decreased capacity of the aged individual to adapt himself to the constant changes in conditions of life.

REFERENCES

BANGA, I. (1953). *Nature, Lond.*, **172,** 1099.

BANGA, I. (1954). *Nature, Lond.*, **174,** 788.

BJÖRKSTEN, J. (1951). *Advanc. Protein Chem.*, **6,** 357.

FREYDBERG, V., and VERZÁR, F. (1956). Symposium on experimental Research on Ageing. Basel: Birkhäuser-Verlag.

HALL, D. A., REED, R., and TUNBRIDGE, R. E. (1952). *Nature, Lond.*, **170,** 264.

HALL, D. A., REED, R., and TUNBRIDGE, R. E. (1955*a*). *Exp. Cell Res.*, **8,** 35.

HALL, D. A., REED, R., and TUNBRIDGE, R. E. (1955*b*). *J. Geront.*, **10,** 388.

HALL, D. A., REED, R., and TUNBRIDGE, R. E. (1956). *Nature, Lond.*, **176,** 966.

HÜGIN, F., and VERZÁR, F. (1956*a*). Symposium on experimental Research on Ageing. Basel: Birkhäuser-Verlag.

HÜGIN, F., and VERZÁR, F. (1956*b*). *Pflüg. Arch. ges. Physiol.*, **262,** 181.

VERZÁR, F. (1955*a*). *III Int. congr. Geront.*, p. 139. Edinburgh: Livingstone.

VERZÁR, F. (1955b). *Experientia*, **11**, 230.
VERZÁR, F. (1955c). *Helv. physiol. Acta*, **13**, C 64.
VERZÁR, F. (1956). *Helv. physiol. Acta*, **14**, 207.
VERZÁR, F., and FLÜCKIGER, E. (1955). *III Int. congr. Geront.*, p. 524. Edinburgh: Livingstone.
VERZÁR-MCDOUGALL, J. (1955). *III Int. congr. Geront.*, p. 247. Edinburgh: Livingstone.
WOOD, G. C. (1954). *Biochim. biophys. Acta*, **15**, 311.

DISCUSSION

Danielli: I find it almost perplexing that the suggestion that the plasticity of the cell—the relative absence of cross-linking—is fundamental to life, should be followed up by this observation of cross-linking in ageing. This is so concrete a fact that it exposes in many ways the bareness of the evidence for my hypothesis. Cross-linking could, as you suggest, conceivably be a fundamental change, perhaps the most fundamental change at the molecular level. Collagen is, of course, an extracellular system. Unfortunately, we know extraordinarily little about the mechanisms of cross-linking in natural molecules. I wonder if anyone feels able to make a comment on cross-linking mechanisms.

Bourne: I do not want to comment on cross-linking mechanisms, but in any collagen fibre there are various types of polysaccharide inside the fibres and between the fibrils, and these must be taken into account in any interpretation of age changes in collagen. I don't know to what extent these play a part in this problem.

Verzár: I was most impressed on hearing from Prof. Haddow* that cross-linking may in some circumstances play a part in carcinogenesis. For our own experimental results we have no other explanation to offer than cross-linking. We do not know whether increase in cross-linking is a general happening in the body. We also do not know why these cross-linked molecules remain cross-linked and are not renewed. There are some ingenious older hypotheses about cross-linking processes in ageing, but at present we can only say that in certain cases we have really proved that this happens.

Comfort: It would be nice to know, Prof. Verzár, whether this occurs in other animals, or in man, so that we could see whether there is any relationship between the time scale of this change and the time scale of the process of senescence.

Verzár: We have tested this only on rats but it is possible that one could start to work it out also for other animals.

Comfort: The tendo Achillis in man should be fairly accessible. I don't know how much surgical material you could get but you could

* This referred to the possible rôle of cross-linking in the carcinogenic action of certain of the biological alkylating agents, notably the nitrogen mustards; and to the question whether such carcinogenic agents merely expedite ageing processes which occur spontaneously in their absence, and which may be responsible for a proportion of so-called spontaneous tumours.—A. HADDOW.

certainly get postmortem material and I should have thought that the ligamentum nuchae in the ox could be obtained.

Tunbridge: There is a very simple evidence—it is not complete—in the human. If you take the tendo Achillis of a foetus or of an infant up to about five or six months, there are marked differences in structure at whatever light level you employ, i.e. the light or electron microscope; the tendon gradually assumes an ordered form with increasing age. One has to be careful about the interpretations of things seen at different light levels, but the physical chemist would accept that the form of the tendon in the foetus is different from that obtained from an infant over the age of six months. It has been suggested that the change arises as a result of walking, but we have found it to be present before six months whether the child walks or not. The tendon seems to retain the morphological appearances obtained at six months throughout the rest of life. Changes do occur but one cannot exclude the possibility that such changes are the result of trauma or disease. One must be very careful of saying that changes, because they are associated with chronological age, are automatically the result of an ageing process. There is evidence for structural changes in young tissue which may have biological significance, but one must not infer that they occur because of ageing.

Verzár: The 5-month-old rat is young, the 10 to 18-month-old one is in the prime of life, from 18 to 24-months old I would call it an old rat and after 24 months it is a senile rat. The rough morphological picture of the tendon fibre is not changed in the rat from the fifth to the twenty-fourth month, and during this time there is continuous change of the force of thermic contraction.

Landowne: We have made some observations on humans which may be relevant here. We used an external stimulus to set up a pressure-wave in a living human brachioradial artery. This has only the advantage that the subject is living at the time, but it has the great disadvantage that we have no dimensional data, so the following is not a physical, but a physiological, study. We were able to relate the pressure existing at the moment to the velocity of propagation of such a pulse-wave (1951, *Fed. Proc.*, **10**, 78; 1952, *J. Geront.*, **7**, 485). I shall ask you to accept the square of the wave velocity as an approximate index of the change in distensibility of the vessel, as pressure changes. With age, the relation between velocity and pressure changes: in the young individual we have a curve concave to the pressure axis; in the older individual the curve tends to be initially higher, but to rise more slowly and to show less curvature. While absolute level changes with age at some pressure levels, it does not change in our material at other pressure levels. This is a study of age clinically, i.e. the agewise differences include all the things that man is heir to, and specifically this includes Mönckeberg's sclerosis. But in speculating upon the possibilities behind this, it appeared that the change in distensibility with a change in stress was less in the old individual and we felt this could be accounted for by an increase in cross-linking or change in the mobility of the long-chain molecules. Here, of course, we are dealing with a very complex structure that has much more than collagen in it.

Baló: I was very interested in hearing Prof. Verzár's report of his finding that in the sciatic nerve heat contraction takes place between 76° and 92°, whereas heat contraction of collagen takes place at a lower temperature; we have found it at 62–70°. This finding suggests that some protein material which belongs to the nerves contracts, and that can only be the axis cylinder. It would be interesting to carry out some work to clarify this problem, because the contraction of collagen has given some insight into the structure of collagen. We have found that contraction of collagen fibres takes place, and that relaxation follows after contraction. Now, at the time of contraction, mucopolysaccharides are dissolved and at the time of relaxation the pro-collagen is dissolved, and we found that, in old age, relaxation does not take place because the pro-collagen (which makes up 20 per cent of the collagen fibres) cannot be dissolved. Since this has given an insight into the structure of collagen, I think that if we could prove that a similar alteration takes place in the nerve fibres then we would have a clear idea of the structure of the axis cylinders.

Nicolaysen: Prof. Verzár, can you take fibres out of the living body (of a young rat, for instance) and preserve them deep-frozen for a couple of years, say, and then test them?

Verzár: I have kept them a month in the ice-chest and they remained the same.

Gillman: In relation to Prof. Verzár's contribution it may be worthwhile thinking of the possibility of testing, by his techniques, alterations in collagen in scars as they age. Admittedly, Abercrombie and co-workers (Abercrombie, M., Flint, M. H., and James, D. W. (1956). *J. Embryol. exp. Morphol.*, **4**, 166) have recently indicated that a wound can undergo contraction in the absence of collagen deposition in scorbutic animals; but at the same time there would seem to be considerable indication that in the latter stages of the healing of certain types of wounds, in humans and rabbits at any rate, there is considerable contracture which seems to be associated with diminishing cellularity in the healing area. In scar tissue in different organs in the body there are morphological and tinctorial changes with the ageing of the scar tissue; apart from the alteration in cellularity (which everyone accepts as one of the phenomena of ageing scars) there are also alterations in the tinctorial reactions indicating the development of fibres which "take" elastic stains. I am not by any means implying that everything that stains with elastic stains is elastic tissue. In fact we have called such altered collagen "pseudo-elastic" tissue (Gillman, T., Penn, J., Bronks, D., and Roux, M. (1955). *A.M.A. Arch. Path.*, **59**, 733). And whether you find such pseudo-elastic tissue in a scar or following chronic injuries (like those in the skin due to ultraviolet or X-ray irradiation), or in an organ like the liver, where long-standing cirrhosis is also associated with an alteration in the collagen which acquires "elastic" staining, or whether in arteries, we have shown that the same thing seems to occur in all instances when collagen ages. One would be interested to know (and I think that Prof. Verzár's methods would give some indication) whether it might be worthwhile, both experimentally and in man, to test the reactivity of collagen in scars

of known ages found in individuals of different ages, i.e. does a young individual lay down collagen of a kind different from the collagen which an old individual lays down under similar experimental conditions? And, in individuals of different ages, does the scar tissue itself age in different ways?

Welford: I should like to comment on the part of Prof. Verzár's talk dealing with change of adaptability with age; and to link this to the discussion of variability with which we began this morning. We, in our studies of behaviour, have found this increase in variability as one goes up the age scale, but the precise form of its change with age has led us to suspect that in a number of cases one is observing a pattern of events somewhat as follows: among the younger age groups potential capacity exceeds the demands of the task; in other words, some factor other than the capacity that one is studying is holding performance constant. The potential capacity is gradually changing, however, with age, and eventually falls to a point at which it becomes the limiting factor. As a result, we have a system in which up to a point one has a limitation imposed by some factor which does not change with age, but beyond this point there is functional dependence upon a capacity which does change. Now, this has two important implications; the first is that if you test performance with a task which stretches even a young person's capacity to the maximum you will get a fall off at an earlier age than if an easier task is used. Secondly, it has an important bearing upon the type and cause of variability that one would expect to find. One normally thinks of increased variability among older people as the result of people ageing at different rates, but this is not the only possible cause. If what I have been saying is true, one would expect to find some factor such as the demands of "reasonable" performance determining the level of performance among the younger subjects, but that as one went up the age scale so the performances of more and more people would be limited by failing capacity. For those of higher initial capacity, however, such limitation would not begin until a relatively late age. Thus, during middle and early old age one might expect a gradually diminishing number of people to maintain a performance comparable with that of young people while others showed more and more profound changes. The increasing variation, however, would not be due to different rates of ageing but to differences of initial capacity existing throughout life, but masked until middle or old age.

Tunbridge: I should like to go back to Prof. Franklin's work on the effect of using a toothbrush. This is a fascinating study not necessarily confined to the problem of ageing. Does he not think that in the human there may be marked differences in the method of brushing unless it is supervised?

There is some evidence that there is a greater tendency for keratinization to take place more readily in mature than in young tissue. Does Prof. Franklin wish to suggest that there is a greater degree of change with increasing age or is he merely re-emphasizing the differences between young and mature tissue?

Franklin: We realize that it is difficult to get patients to brush in a completely standard fashion, but we are taking preliminary steps to

overcome the difficulty. Already in the human subject, entirely apart from ageing, we have got some rather spectacular cures.

Tunbridge: It is an interesting sideline, that keratinization gives protection against infection.

Franklin: In some of these people, even with one month's brushing, the infection clears up.

Gillman: I don't think that the occurrence of keratinization can always be related to the degree of resistance to infection. For example, in vitamin A deficiency and also in pellagra, excessive keratinization is associated with increased susceptibility to infection. On the other hand, keratinization, or its equivalent, in the vagina seems to provide resistance to infections.

Franklin: I can only say that those are two clashing facts which we will attempt to elucidate later. In any case, the main object, to my mind, is not to help the dental work but to find something that changes with age and so far, to a slight degree, the effects of this brushing have changed with age.

Lorge: The obviously very limited data we have on the human are extraordinarily interesting. I should like to raise one minor question which again relates to American dental experiences: that is that the amount of pressure or amount of weight that is given to the brush stroke by a youngster as opposed to an adult may be significant. Another point is: what kind of brush stroke produces what kind of effect? It may be interesting to see whether one can measure the amount of weight or pressure exerted against the gums by people of different ages, just to see how much it is. It would be interesting to correlate, for instance, the strength of grip with the result. If you were able to correlate dynamometer strength with your results, you may have some additional evidence.

Tunbridge: I feel from experience of trying to teach patients to do things that the degree of error in any technique so taught is enormous. However capable one may be as an instructor—and obviously the more effective your personality the greater the degree of initial success—only some 15 per cent will continue to do as you have asked them if the process is to be continued for a long time. Might it not be better to choose three age groups? One might take a residential school for children, a prison for your middle aged group, and an Old People's Home. If you were to take twenty people in each of these groups and to use a dynamometer brush you might be able to standardize the technique and, insofar as the samples are fair representations, obtain a quicker answer to your problem than with the present methods.

Lorge: When we talked of going into World War II, the United States War Department asked me to standardize an intelligence test. When I proposed to use prison populations, the study was rejected on the grounds of "What will the mothers of our soldiers say when they realize that the tests that we used and the results and the findings come from prisons, and not from normal adults!"

Bourne: In this country, if you use public schools and prisons, you get comparable diets, of course!

Franklin: Is this inside information?

METHODS AND LIMITATIONS IN STUDIES OF HUMAN ORGAN SYSTEM FUNCTION

Milton Landowne

Gerontology Branch, National Heart Institute, PHS, D.H.E. & W., Bethesda, and the Baltimore City Hospitals, Baltimore, Maryland

Experiments where time is a variable are so essential to most researches that one could fairly include all scientific disciplines and methods in a discussion of the methodology of ageing. When the primary focus is upon ageing or age changes in human biology, where studies may extend from cytological to anthropological areas, certain modes of approach appear to be more inviting or fruitful, and others appear formidable or limited. Rather than essay a categorical review of methods which have been used in the study of age changes in man, I should like to discuss some of the approaches and share some of the limitations of methodology with which we have been concerned in our studies of physiological function in contemporary adults. The word "contemporary" emphasizes not only that we lack longitudinal studies for the most part, but also that the samples of the species which we study yield us data about how man today has aged and not whether man ages apart from his present environment. This, then, is a pragmatic view of ageing; and, in this, all things which alter structure and function with time are relevant and are to be counted. While our task is certainly to describe the changes, it is important that we do so in a manner which attempts to identify the nature and the cause of change, or to learn how change is avoided.

Paramount to our undertaking is the method of sampling. Although we measure individuals, our results and conclusions have tended to describe the group rather than the individual. If it were at all possible to have a "pure" sample of entirely

"normal" man, or for that matter even a good sample, then one might cautiously venture to propose that agewise changes in such a sample were manifestations of normal ageing and, therefore, these might constitute the indices and the representations of the normal ageing process. The unfortunate fact is that we use the word "normal" here to mean "without abnormality" or "free of pathology", etc., and not in its alternate statistical sense of "norm", referring to the common model, the standard or the representative type. We may try to obtain our group of "pure" normals by excluding known or testable abnormality, but since we can hardly exclude unknown abnormality, we are unable to apply a criterion of the goodness of our selection, and have no test of purity. If all the subjects in an experiment are represented by 100 per cent, such diseased subjects as may be readily identified might constitute, let us say, about 10 per cent of the population. This itself might not be too disturbing, but an increasing agewise incidence of this fraction does disturb us. We may deal with this by excluding the members of this group in order to obtain greater uniformity and less interfering disease. But do we succeed in this? Although manifest disease may be readily diagnosed, minimal to moderate disease is sometimes found in higher incidence than can be reliably disclosed by our clinical methods of examination. Fig. 1, from the data of Willius, Smith and Sprague (1933), shows the percentage incidence of coronary arteriosclerosis in a series of over 3,000 consecutive male autopsies at the Mayo Clinic. The incidence of marked and of extreme disease is of an order compatible with clinical figures, but only 2 out of 1,000 subjects 60 years or older were judged free of the disease on gross morphological examination of the heart. On this basis, an individual of 60, without coronary arteriosclerosis, is abnormal; i.e., he has a statistically improbable condition—he is unusual. Our task is to determine whether cardiac functional changes are in consequence of or despite coronary sclerosis; to ascertain what effects and interpretation these changes may call for. This is not simple. To illustrate, the electrocardiographic

response to a standard exercise (Master and Oppenheimer, 1929) was ostensibly designed to disclose coronary arteriosclerosis in individuals with equivocal or no clinical evidences of cardiac disease and in whom the electrocardiogram at rest was normal. Agewise differences in the normal electrocardiographic response to exercise have been reported by our laboratory (Silver and Landowne, 1953), as well as by others

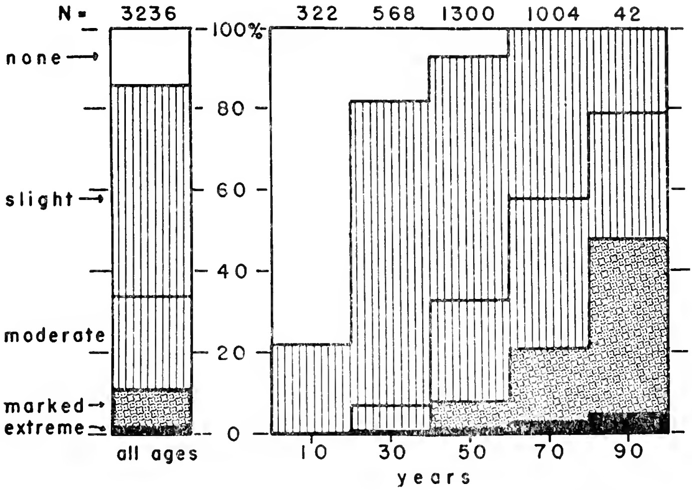


FIG. 1. Gross morphological total and agewise incidence of coronary sclerosis, graded as to severity and expressed as percentage of the corresponding age group. Data from over 3,000 consecutive male autopsies reported by Willius, Smith and Sprague (1933). The number of cases in each age group is indicated at top of the column.

(Master, Friedman and Dack, 1942; Simonson and Keys, 1956). Although there may have been important differences in the manner and effectiveness of selection of these "normals", it would have to be admitted that they do not represent "pure" normals, but are carefully selected subjects in whom, statistically, coronary sclerosis is present with increasing agewise incidence and severity. To have obtained a lesser contaminating incidence of disease, one would need to have known at least the information which was supposed to result

from the study. It is therefore plausible to consider that certain of the agewise changes in the "normal" response are due to disease; and, as a result, the test indulges an increasing tolerance in the clinical evaluation of older subjects. A similar picture exists for the general as well as the cardiac arterial circulation. Fig. 2 represents data for arteriosclerosis of the aorta from the Mayo Clinic experience (Willius, Smith and Sprague, 1933). While general morbidity statistics do not

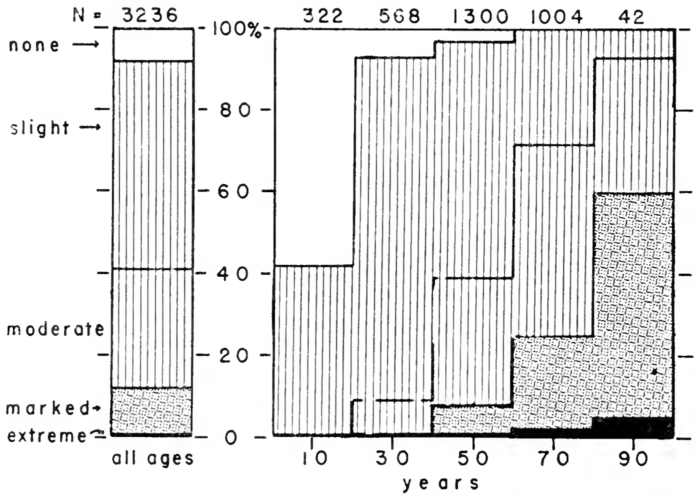


FIG. 2. Gross morphological total and agewise incidence of aortic sclerosis, graded as to severity. Source and legend as in Fig. 1.

reveal arterial disease in the extremities to be extremely common, this refers to clinical and not to latent disease; and clinical manifestations are commonly complications or sequelae. The incidence of sub-clinical disease might tell the same story as do the figures for aortic involvement.

Parenthetically, the arterial disease referred to is mainly atherosclerosis. In concept, this may exist as a process or tendency well in advance of even subtle morphological, physical or chemical indices. Thus, even when we shall have the diagnostic techniques to detect arterioscleroses before

limitations of arterial function compromise the function of the tissues served, disease may be in a well established stage.

In the area of cardiovascular physiology then, the contemporary pattern of early and increasing age incidence of atherosclerosis makes the possibility of selecting "pure" normal subjects remote, if by "normal" we mean free of this disease. Moreover, for the time being, the dependence of man on his vascular system places the burden of proof upon any hypothesis which does not consider the rôle of the circulation in relation to age changes in any tissue. To a greater or less degree, the agewise changes which have been described in renal function (Shock, 1952), glucose tolerance (Silverstone *et al.*, 1956), nerve conduction velocity (Norris, Shock and Wagman, 1952), etc., might be secondary to vascular alteration. Before proposing that these agewise effects represent primary manifestations of ageing, the rôle of the circulation is to be considered.

It would be much simpler to select a representative sample of a population, and, in this manner, give a picture of the "norm". This would be descriptively acceptable. Severely limiting the success of this approach are those areas where the degree of abnormality will hardly remain constant over any age span, and much of our variance becomes ascribable to the increasing age incidence of pathology. A recent study of pulmonary ventilatory function (Norris *et al.*, 1956) may be used to emphasize the double quandary, and here disease of more than one system may be present to affect pulmonary bellows function. Fig. 3 indicates the incidence of clinically diagnosed conditions. The incidence of acute and severe chronic pulmonary disease is approximated, for such subjects were not included in this study; we have actually practised selection in all, or almost all, of our studies. Agewise increase is noted in several relevant diagrammed disorders, and multiple disorders commonly coexist. If the severely ill subjects had been included in this study, we should have had no age study, but a study of disease. On the other hand, if all cases

suggestive of disease had been excluded, we should have few and insufficient old subjects.

In some studies with few subjects, we have described individual results, still hoping that we had selected the

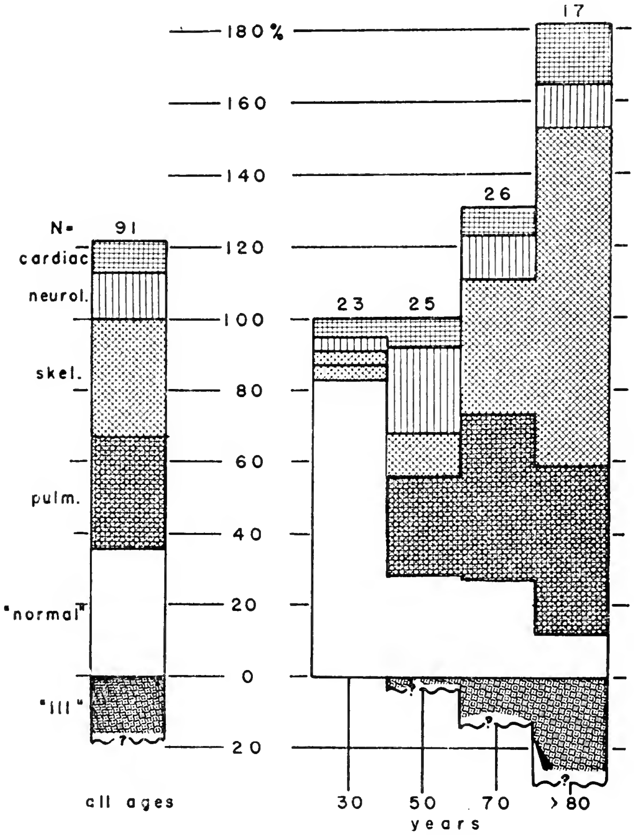


FIG. 3. Incidence of certain categories of diagnoses, expressed as percentage of the total and agewise display, for 91 subjects of a pulmonary function study. The incidence of acute or severe chronic pulmonary disease was not recorded and is only suggested.

"normal" or the "norm". These were detailed metabolic balance studies such as ACTH studies (Duncan *et al.*, 1952) or calcium balance studies (Bogdonoff, Shock and Nichols, 1953).

There is, however, much of additional value to be gained by observation of the unique. Dr. Dickinson Richards has a recent paper on the ageing lung which includes the consideration of a few highly selected subjects with no taint of disease. Such few unique individuals deserve most careful study.

I believe the most useful information is obtained not by any single one of the sampling methods, but with all of them, singly and together. One may successfully weigh and find inconsequential the influence of a small percentage of contamination with diagnosed or undiagnosed disorders. One may even deal with a sizeable percentage if agewise bias can be excluded or accounted for. Wherever disease is dominant, any known limitation of the clinical art ought to be fairly recognized, for clinical authority has been defined as "the obsessed state" and the specificity of clinical diagnosis may have a wide range of variation. My approach does not mean at all to deprecate clinical evaluation, or to devalue studies on older humans because there may be a high incidence of pathology in clinical or putative normals. Such studies and evaluations are needed, but needed too is cognizance of the limitations of the material. It may be pointed out that this problem in subject selection applies as well to age studies in other species than man; but the science, as well as the art, of clinical diagnosis has reached higher levels in the human.

Many of the studies which may be performed in humans do not yield information with a highly specific meaning and, thus, the interpretations of experiments which reveal age differences may be difficult. As examples, the age changes in vitamin B_{12} excretion after intramuscular injection (Watkin *et al.*, 1953) or in uric acid excretion after ACTH (Solomon and Shock, 1950) may be the consequence of renal functional change rather than of vitamin deficiency or adrenal functional decline. Although an electrocardiogram appears to be a specific cardiac record because it registers electrical potentials of cardiac origin, this is not quite the case; and particularly, it is not a specific indicator of myocardial function. These observations are nevertheless valuable, and valid conclusions may be reached

particularly by the addition of relevant and critical evidences from several different points of view.

As an illustration of a quasi-specific study, I might outline the approach and results of some observations of skin temperature which we have made (Landowne, Silver and Silverstone, 1954). The physiological literature refers to statements that skin temperature is higher during the more active and robust years and decreases with advancing age, but it does not yield critical data in this regard. Because of the reduction in basal metabolism with age, the older subject loses less heat to his environment. It is then to be expected that a reduction occurs in the heat transferred by the blood flow to the skin. The popular concept and interpretation of subjectively cooler extremities in some older individuals is that of a reduced resting peripheral circulation. Agewise changes in cutaneous circulation might alter the efficiency of body temperature regulation and might also underlie the age changes in structure and functions of the skin. In moderately cool, still air at constant temperature, heat losses by convection and evaporation are minimized and the total heat loss, which occurs chiefly by radiation, is proportional to the skin temperature. In this manner, the skin temperature can provide an index of the cutaneous circulation and of the heat loss under resting conditions. In addition, under conditions of induced vasodilatation, the skin temperature can yield information as to the functional integrity and reserve of major arteries, as well as of the cutaneous blood supply. The analysis of the temperature response of the skin to heating of the trunk is taken to be an indication of the maximal blood flow to the skin. The maximal cutaneous blood flow is, in turn, considered to reflect the minimal vascular resistance of the skin circulation, and by presumption, on the average, of the circulation in the entire limb. The commonly encountered cause of fixed increase in vascular resistance (except in hypertensive subjects) is arteriosclerosis of the major arteries. The portion of the total regional or planetary population available for investigation (Fig. 4) may be considered to include an undetermined and

unidentified, but statistically probable, number of cases of peripheral arteriosclerosis. To this group, certain arbitrary criteria are applied and a sample is selected for study. Either

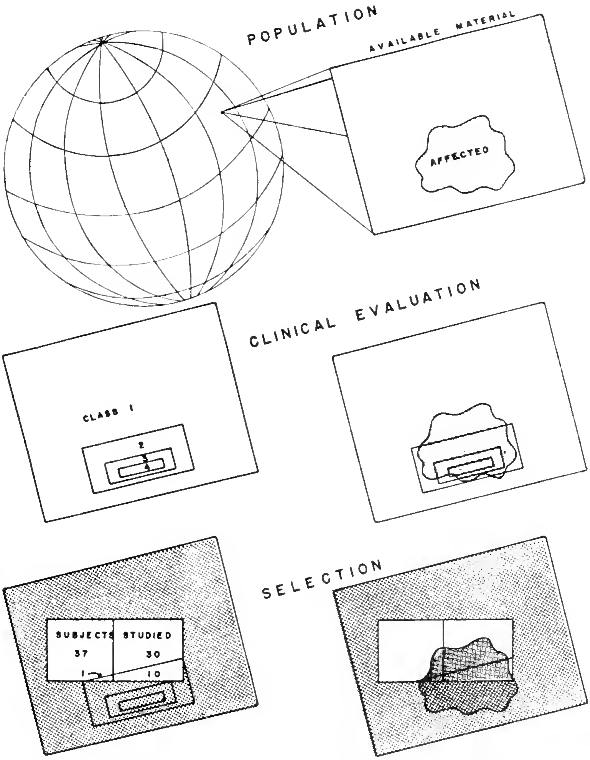


FIG. 4. Schematic method of obtaining a study sample. Individuals affected by disease are shown as if they could be gathered within a shaded area; clinical classifications are indicated as within successive rectangles, in order to show that there is overlap and disparity between disease and its clinical recognition. In the sample selected for study, the relation between clinical classification and disease identification may not be the same for young and old subjects.

by deliberate inclusion or by failure of exclusion, a number of cases possessing arteriosclerosis will be present in our final selected sample. Out of a fairly large number of subjects

available for study, a group of 78 were selected as suitable; their ages ranged from 26 to 93, with a mean of 58. They were chosen because they presented no disorder which we thought would interfere with the test or its interpretation, and because they had no symptoms to indicate the existence of peripheral arterial disease. Eleven of these subjects were considered to warrant functional classification according to the American Heart Association standards as class 2 (minimal impairment in reserve), and the remainder as class 1 (no impairment). Each subject was studied twice. The skin temperature was obtained at six sites by multiple point automatic recording thermocouples. After equilibration for 90 minutes in a room constant at 22·7° C, reflex vasodilatation was induced by specially designed radiant heating applied to the trunk and abdomen. This method of eliciting a maximal vasodilatation in fingers and toes was selected as convenient, efficient, and well tolerated. Subject acceptance of procedure is, of course, a prime requisite of experimental design.

From a study such as this, we have come to these conclusions: under standard conditions, these 78 subjects, without evident peripheral vascular disorder, showed a uniform temperature of the fingers and a slight but statistically significant agewise increase in the temperature of the toes. The observation that older subjects have warmer toes, under these conditions, does not support the idea of any decrease in resting skin flow with age. Since blood flow is relatively low under these conditions, and skin temperature is only an indirect index of flow, it cannot be concluded that resting flow to the toes increases with age. In some older subjects, toe temperature exceeded finger temperature, showing a reversal of the finger-toe gradient which is considered to characterize the relative vaso-motor participation of the upper and lower extremities in heat regulation. These are evidences for a change with age in the differential heat-regulating activity of the upper and lower extremities. There may, therefore, be a greater relative heat loss by radiation in the toes of older individuals at rest in a cool environment, due either to a greater

total heat loss or else to compensation for a reduction in the evaporative heat loss. In response to a vasodilating manoeuvre, the temperature rose in the fingers and in the toes in most subjects, but in a varying degree and manner.

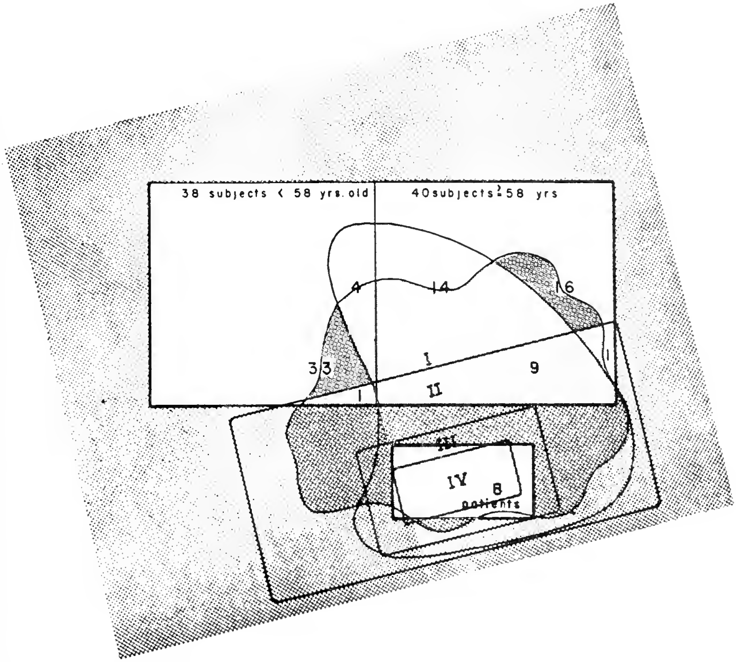


FIG. 5. Schema of overlap of hypothetical "true" disease incidence (irregular dark shaded area), clinical classification (tilted rectangles numbered I—IV), and boundary (free form figure) indicating a particular test response or performance level which, in this case, is 30·7° C. Seventy-eight subjects and 8 patients are represented by the two heavy bordered rectangles; the distribution of "positive" and "negative" tests is indicated for the subjects, divided into two age groups.

For the toes (Fig. 5), the response of subjects between 40 and 58 years of age was uniformly good. The average response of younger subjects was even better, although several younger subjects showed little rise. Some old subjects obtained as high a temperature as did some young subjects, but there was an overall significant decrement in the average response with

age. In over one-third of all 78 subjects, the toe temperature did not exceed the group mean of 30·7° C. Among the 40 subjects who were older than the mean age of 58, two-thirds did not attain this temperature. Nine of the 11 subjects who showed minimal clinical evidence of functional impairment had responses to less than mean values, but there were 18 subjects clinically graded as class 1 whose responses were no better. The response of 8 patients with manifest and advanced disease of classes 3 and 4 have been added. In none of these did the toe temperature exceed 30° C. However, they did as well as 10 of the class 1 subjects in the same age range. This might suggest that the test procedure lacks sensitivity or selectivity. It may alternatively indicate that the functional capacity of subjects and patients was about equally limited. In the patients with manifest disease, symptoms and signs had developed, while in the subjects who were not patients, these complications of a latent disorder had not occurred. Although useful, a single test of this kind cannot be considered to establish a diagnosis without other corroborative evidence. To be a good diagnostic procedure, a single test should provide a small number of false positives and false negatives, but as long as the incidence and distribution of the cases of disease are not known with certainty, we cannot determine the accuracy of detection by any proposed index; this includes our own quasi-specific measurements.

Somewhat more may be ventured in the way of interpreting agewise changes in studies which deal with more specific measurements, particularly if these are combined with other equally or less specific measures in the same individual, or in similar groups. This may be illustrated by the cardiac output studies which we have made (Brandfonbrener, Landowne and Shock, 1955). It might have been expected that we should find cardiac output to decrease with age. Earlier work suggested it. It had also been demonstrated, or indicated to be likely, that regional increases in the renal, cerebral and extremity vascular resistance occurred with age. This would lead to an increase in total resistance of the greater circulation, a

significant redistribution of this resistance, or both. Especially in association with a decrease in cardiac output, an agewise increase in mean arterial pressure inferred from systolic and diastolic values (Master, Dublin and Marks, 1950) would indicate an increase in total resistance; thus, the interrelationship of output and pressure provides for an evaluation

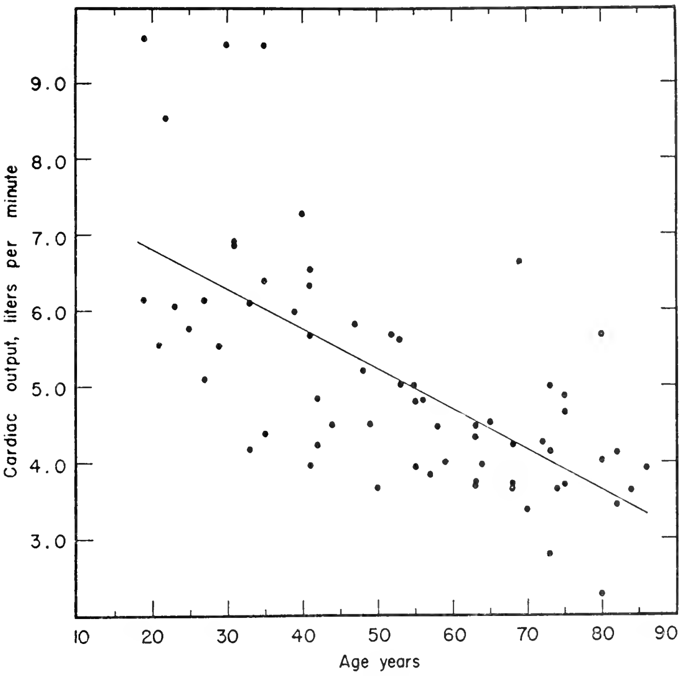


FIG. 6. The relation between cardiac output and age in 67 "normal" men.

of peripheral circulatory performance. Determinations of cardiac output were made in 67 males between the ages of 19 and 86 years of age by an indicator dilution method. According to clinical criteria, these subjects were free of suspected relevant disease, and they form the basis for an agewise analysis of change. The basal cardiac output averaged 5·08 l./min. with a standard deviation of 1·51 l./min. Fig. 6

shows a scatter plot of the individual values. The coefficient of variability was $\pm$ 32 per cent. About half of the total variance, or one-third of the standard deviation, turns out to be associated with difference in age. A decline in cardiac output is evident after the third decade, and, on the average, there is a decrease in cardiac output of 1 per cent of the 50-year value per year.*

Table I

PARAMETERS OF REGRESSION EQUATIONS BASED ON THE FORMULA:

$$y = y_{50}\, e^{(b \pm S.E._b)x}$$

WHERE y_{50} = PREDICTED 50-YEAR VALUE; b = PERCENT CHANGE PER YEAR; $S.E._b$ = STANDARD ERROR OF b, x = AGE MINUS 50 YEARS.

Item	*Average at 50 years*	*Average Change (%/yr. ± S.E.)*
Cardiac output (l./min.)	5·01	$-$ 1·01 $\pm$ 0·13
Cardiac index (l./min. m.^{2})	2·91	$-$ 0·79 $\pm$ 0·13
Surface area (m.^{2})	1·72	$-$ 0·22 $\pm$ 0·06
Heart rate (beats/min.)	68·7	$-$ 0·31 $\pm$ 0·11
Stroke index (ml./beat m.^{2})	42·3	$-$ 0·49 $\pm$ 0·13

The 1 per cent decrease in cardiac output per year may be seen (Table I) to be due in most part to a decline in cardiac

* In Figs. 6 and 7, the line represents a simple linear regression equation with a slope that is significantly different from zero. The equation $y = a + bx$, and the standard error of the slope b is a conventional and convenient way of expressing results. The assessment of relative contributions of several variables may be furthered by using partial regression statistics. Slight modification of this representation is even more appropriate to these agewise studies. The linear regression equations fitted to the natural logarithm of values for cardiac output, etc., furnishes an equation of the form $y = ae^{bx}$. The fit has been generally improved by this, and that technically justifies the procedure, but we do not suppose that this actually represents the true regression pattern. The second equation possesses the truly added advantage of providing a series of dimensionally consistent expressions, for "b" is now the fractional change per year. When "x" = age in years, "a" becomes the predicted value at zero years of age which is a relatively useless bit of information. But by making "x" equal to the difference between age and 50 years, "a" becomes the predicted 50-year value. This is more informative than is the mean of the sample, and serves as a mild emphasis to give the older individual his due.

index by 0·8 per cent per year, and to a slight reduction in calculated surface area. The regression of cardiac index on age is due partly to an average decrease in heart rate averaging 0·3 per cent per year, while the remainder, or 0·5 per cent per year, represents a reduction in stroke index, the blood pumped per beat per unit of body size (Fig. 7).

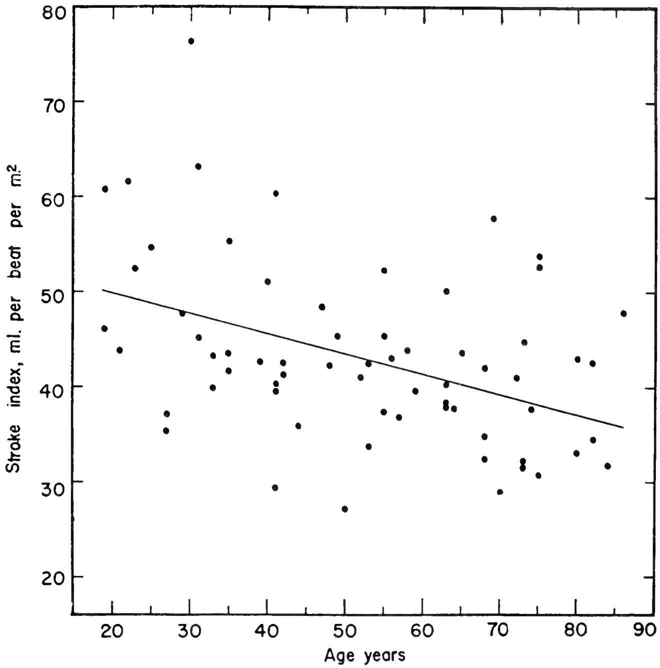


FIG. 7. The relation between cardiac stroke index and age for 67 "normal" men.

Blood pressure was measured in these subjects by brachial intra-arterial needle and the mean blood pressure was found to increase by only 0·1 per cent per year. Therefore, the decrease in cardiac output was in association with an increase in pressure flow ratio, the vascular resistance, which averaged 1·1 $\pm$ 0·14 per cent per year. The change in output and resistance are greater than the rates calculated (Landowne,

Brandfonbrener and Shock, 1955) in similar manner for the average decrease with age in total oxygen consumption, and carbon dioxide production (Shock and Yiengst, 1955), or for estimates of total intracellular water (Shock, Watkin and Yiengst, 1955), based on data which have been reported for similar but less rigidly selected groups of subjects in studies from our laboratory (Table II). These indices all decrease by

Table II

THE RELATION BETWEEN CARDIAC STROKE INDEX AND AGE FOR 67 "NORMAL" MEN. COMPARISON OF 2 GROUPS OF SUBJECTS SUGGESTS THAT THE INCREASE IN VASCULAR RESISTANCE EXCEEDS THE DECREASE IN INDICES OF OVERALL METABOLISM.

Item	*Average at 50 years*	*Average Change (%/yr. ± S.E.)*
Resistance (mm. Hg min./l.)	18·0	1·11 ± 0·14
O_2 uptake (ml./min.)	212	− 0·53 ± 0·05
CO_2 production (ml./min.)	172	− 0·53 ± 0·07
"Cell water" (litres)	16·5	− 0·58 ± 0·12

less than 0·6 ± 0·12 per cent per year. Considering these items as general indices of the reduction in metabolically active tissue, the greater average increase in vascular resistance indicates a reduction in the generosity of cellular perfusion. This further suggests that overall, and on the average, the changes in metabolic activity and protoplasmic mass may be consequences of circulatory attrition; for if these changes occurred *pari passu*, or if cellular alterations were to be considered primary to reduction in circulation, it would not be likely that the decrease in perfusion would exceed evidences of reduction in cellular activity. These evidences for primary vascular alteration are intended to apply to the total circulation generally, for in any one circulatory region, circumstances may be quite different. From studies on renal blood flow (Davies and Shock, 1950), and resistance (Landowne and Shock, 1951), which have been carried out in our laboratory,

the average decrease per year in renal perfusion turns out to represent more than half of the total average reduction in cardiac output, although the kidneys in a young adult receive less than one-fourth of the total flow. The increase in renal resistance with age is, therefore, more striking than is the increase, on the average, of the non-renal portion of the greater circulation. Consequently, the changes in renal resistance contribute in good measure to the overall disproportionality which we have deduced between decreased perfusion and diminished oxygen uptake. Not only each circulatory segment but each metabolite may contribute to the overall picture in a different fashion. The older individual therefore not only has a reduced total circulation, but, logistically, has altered the distribution of blood to various organs. It is tempting to speculate teleologically that he reduces circulation in regions where it can be more readily reduced, but this finds him less prepared to cope with urgent or sustained needs. This concept is supported by our estimates that, despite an increased fixed renal resistance, there may be no reduction in renal vasoconstriction in the older subject (Landowne and Shock, 1951).

From the observations on cardiac output and blood pressure, we have also drawn deductions indicating that there is a decrease in the work of the left ventricle, and in the rate at which this work, on the average, is performed; i.e., in the "power" (Landowne, Brandfonbrener and Shock, 1955). The decrease in cardiac output and work need not be considered as evidence that the older subjects had subclinical heart disease, since, in compensated heart disease, normal or even high values may be obtained. It appears more likely that the decrease in cardiac output is related, in part at least, to alterations in the circulatory requirements of older people at rest. As a byproduct of a loss of circulatory pathways, the demands upon the pump are reduced. Cardiac size, however, does not decrease—thus, the heart is larger in relationship to body size and to cardiac work. This relative increase in heart size, the decrease in power, and an increase in the

pressure component of left ventricular work is evidence that, despite the lesser requirement for blood, there is a reduction in the effective reserve of the left heart as a muscle pump.

The foregoing has been painted with a wide brush to reflect trends which are demonstrable among groups of ostensibly well people. It is offered to illustrate some of the experimental and statistical methods which we have applied, and the interpretations which endeavour to give us some insight into the logistics of the circulation. In presenting these examples of studies in human cardiovascular physiology, I may have selected a field where dissociation of the progress of years from a high and increasing degree of pathology is currently most difficult. Perhaps I have deliberately laid emphasis on this because I believe cognizance of the limitations, as well as the advantages of the clinical approach, is an asset to inquiry. For the restricted area of organ function study, this report has been an effort to illustrate the reasoning which motivates specific and quasi-specific methods of study.

REFERENCES

BOGDONOFF, M. D., SHOCK, N. W., and NICHOLS, M. P. (1953). *J. Geront.*, **8**, 272.

BRANDFONBRENER, M., LANDOWNE, M., and SHOCK, N. W. (1955). *Circulation*, **4**, 557.

DAVIES, D. F., and SHOCK, N. W. (1950). *J. clin. Invest.*, **29**.

DUNCAN, L. E., SOLOMON, D. H., ROSENBERG, E. K., NICHOLS, M. P., and SHOCK, N. W. (1952). *J. Geront.*, **7**, 351.

LANDOWNE, M., BRANDFONBRENER, M., and SHOCK, N. W. (1955). *Circulation*, **4**, 567.

LANDOWNE, M., and SHOCK, N. W. (1951). *J. Geront.*, **6**, 334.

LANDOWNE, M., SILVER, H. M., and SILVERSTONE, F. A. (1954). II World Congr. Cardiology, and 27th Annu. sci. Sess. Amer. Heart Ass., Part II, p. 441.

MASTER, A. M., DUBLIN, L. I., and MARKS, H. (1950). *J. Amer. med. Ass.*, **143**, 1464.

MASTER, A. M., FRIEDMAN, R., and DACK, S. (1942). *Amer. Heart J.*, **24**, 777.

MASTER, A. M., and OPPENHEIMER, E. T. (1929). *Amer. J. med. Sci.*, **177**, 223.

NORRIS, A. H., SHOCK, N. W., LANDOWNE, M., and FALZONE, J. A., JR. (1956). *J. Geront.*, **11**, 379.

NORRIS, A. H., SHOCK, N. W., and WAGMAN, I. H. (1952). *J. Geront.*, **7**, 489.

RICHARDS, D. W. (1956). *Bull. N. Y. Acad. Med.*, **32**, 407.

SHOCK, N. W. (1952). *In* Cowdry's Problems of Ageing, p. 614. Ed. A. I. Lansing. Baltimore: Williams & Wilkins.

SHOCK, N. W., WATKIN, D. M., and YIENGST, M. J. (1955). *In* Rep. III Congr. Int. Ass. Geront., Old Age in the Modern World, p. 127. Edinburgh: Livingstone.

SHOCK, N. W., and YIENGST, M. J. (1955). *J. Geront.*, **10**, 31.

SILVER, H. M., and LANDOWNE, M. (1953). *Circulation*, **8**, 510.

SILVERSTONE, F. A., BRANDFONBRENER, M., SHOCK, N. W., and YIENGST, M. J. (1956). *Fed. Proc.*, **15**, 171.

SIMONSON, E., and KEYS, A. (1956). *Amer. Heart J.*, **52**, 83.

SOLOMON, D. H., and SHOCK, N. W. (1950). *J. Geront.*, **5**, 302.

WATKIN, D. M., LANG, C. A., CHOW, B. F., and SHOCK, N. W. (1953). *J. Nutrit.*, **50**, 341.

WILLIUS, F. A., SMITH, H. L., and SPRAGUE, P. H. (1933). *Proc. Mayo Clin.*, **8**, 140.

[Discussion of this paper was postponed until after the paper by Prof. Tunbridge.—EDS.]

COMPARATIVE VALUES OF STUDIES OF THE WHOLE ORGANISM AND OF WHOLE TISSUE; CLINICOPATHOLOGICAL TESTS OF AGEING

R. E. Tunbridge

Department of Medicine, University of Leeds

Clinicians, with the possible exception of the paediatricians and the obstetricians, are inevitably concerned with the disorders of the aged. Often they are so near in age to their patients that they are unable to make an objective assessment of the changes which might be associated with increasing longevity. The approach of a clinician is essentially individualistic both from his own and from the patient's viewpoint and has all the bias of such an approach. Nevertheless, the clinician is called upon to assess the effects of heredity, of environment and of disease upon the patient's health, and frequently resorts to ageing as one of the contributory factors, since certain diseases are more prevalent in the aged and they tend to pursue a different course from that followed in younger subjects. Certain pathological changes, e.g. atheroma, arteriosclerosis and cancer which are more prevalent during the second half of life, are often referred to as disorders of old age, yet there is little to justify the vast literature that purports no more than this.

The physician, and, for that matter the layman, is continually faced with the problem "Why do people die?" Particularly is this the case amongst the elderly. Vital statistics tell us that a certain percentage die from cancer, a certain percentage from arterial disease and a certain percentage from infection, etc. The relative percentages vary with the age group studied, but this information does not help in deciding the cause of death of an individual. We have all no doubt known octogenarians apparently full of physical and

mental vigour who died suddenly, yet both the pathologists and clinicians have found it difficult to name the cause of death. Conversely, apparently severe degrees of tissue or organ damage may be found on routine pathological examination without any history of an equivalent degree of dysfunction during life. Perhaps I can best illustrate this by quoting the paper of Vischer and Roulet (1952) who reported the autopsy findings of two centenarians who had lived active, healthy lives free from disability until the onset of a brief terminal illness. The male, aged 102, died with gangrene of the left foot, but autopsy revealed severe generalized atherosclerosis, thrombosis of the left femoral, popliteal and tibial arteries and of the accompanying veins, terminal bronchopneumonia, carcinoid tumour of the ileum, enlargement of the prostate gland, nodular hyperplasia of the thyroid and chronic emphysema. The second case, a female aged 102, died as a result of bronchopneumonia, but autopsy also revealed the presence of severe atherosclerosis with obstruction of the coronary arteries, cholelithiasis, nodular goitre, and severe degenerative changes in the liver and in the heart.

Surveys of autopsy findings in elderly persons by Howell and Piggott (1949, 1950, 1951, 1952, 1953) and Cameron (1955) revealed the multiplicity of the pathological findings observed at autopsy in the elderly. One disorder was the terminal cause of death but many other morbid changes had undoubtedly contributed.

There is general agreement amongst clinicians and pathologists that widespread and extensive arterial changes, particularly atheromatous degeneration, are a common finding in the elderly and the pathological studies I have referred to stress the apparent correlation, but Cameron (1955) is cautious in his conclusions and states: "I doubt very much whether there are specific structural changes due to old age and that alone. I hold the view that ageing is merely the vector sum of a number of morbid processes, most of which take time to develop, and often a long time to reach a serious climax."

Numerous reports have been published during the past twenty years, attempting to correlate the values for certain functions—renal, cardiovascular, respiratory—with chronological age. Prof. Verzár and Dr. Landowne have already referred to the results obtained by the use of some such tests. Clinicians and pathologists have also reported variations in the values for many of the principal constituents of the blood with age. There are many potential fallacies in such reports, those due to the method of sampling, to the interpretation of the norm and to errors inherent in the technique. Many of these points have already been referred to in this symposium and I do not wish to labour them. The matter of the sample is most important and so rarely has a truly representative sample of the total population been studied. The recent survey by Hobson and Pemberton (1955) was based on what the statisticians would accept as a fairly reliable cross-section of the city population of Sheffield. These workers attempted a correlation of the haemoglobin levels, blood urea, blood cholesterol, serum calcium and serum alkaline phosphatase with chronological age. The results for the serum alkaline phosphatase illustrate the inherent difficulties in interpreting results and the difficulty of assessing the norm. The findings were very closely grouped, with the exception of eleven, seven males and four females, in all of whom the value was abnormally high. In eight of these individuals, six males and two females, further investigation revealed that they were suffering from osteitis deformans (Paget's disease). No specific cause was found for the high value in the remaining three patients but it does not necessarily follow that the latter were not suffering from some disease in a clinically quiescent phase or from a disability for which they had been able in part to compensate.

There is always the difficulty of deciding what is normal, and this criticism must be applied to the majority of the clinical surveys of ageing populations. Certain pioneer surveys, such as the survey of Wolverhampton by Sheldon (1948), were invaluable in focussing attention upon the medical

problems of the aged and in providing the politicians and administrators with factual information concerning a cross-section of the population. The survey undertaken by van Zonneveld (1955) in Groningen was even more valuable in that a complete physical examination was included. The survey at present being undertaken in Holland under the direction of van Zonneveld is still more comprehensive since it covers the whole country and includes, besides a full medical and social history, a complete physical examination and an assessment of mental function. Such comparative studies of the whole human organism provide valuable information as to the probable proportions of the population at say 60, 70, or 80 + who are partially sighted, blind, deaf, unable to look after themselves, bedridden etc. The defects named, however, are all readily measurable, but it does not follow that all abnormalities have been detected. Moreover, these studies are horizontal studies and must inevitably include many subjects suffering from disease, albeit unrecognized. What are needed are longitudinal studies in which individuals or a group of individuals are continuously observed over many years, and preferably for their whole life, in order that the rôle of heredity, environment and disease may be properly assessed. Limited longitudinal studies have been undertaken in the case of certain specific diseases, which have added greatly to our knowledge of the natural history of disease. In the matter of ageing it would seem that there are very many inherent difficulties even in a longitudinal study. There is not only difficulty of maintaining constant standards with changing personnel but also the constant pressure to revise methods, the effect of changing environment on the sample, it being impossible to maintain the environmental factors constant and also the effect of increasing knowledge in extending diagnostic accuracy. Some value might be obtained from longitudinal studies such as the continuation of the studies initiated by the late Sir James Spence in Newcastle (Spence *et al.*, 1954) and more particularly with regard to heredity through a study of homologous twins.

Organs and tissues vary both in their complexity and their accessibility. They tend, however, to be less complex than the whole organism and in some cases portions may be detached for detailed study and therefore become much more suitable for controlled studies. All would agree with the observations of Prof. Danielli, that even the simplest tissue is very complex when compared with the cell.

The ease of access of the skin has made it a favoured organ for study. Samples may be readily taken for detailed examination and environmental conditions, in so far as the external surface is concerned, can be controlled. Many changes have been observed in the skin associated with chronological age and even poets and authors have felt the changes sufficiently significant to write about them. All are familiar with the increase of capillary naevi in the elderly, the changes in certain of the skin appendages such as the hair, and the changes in the texture of the skin, the thinning of the skin, the tendency to scarring, haemorrhage and pigmentation particularly in certain sites, such as the face and the extensor surface of the forearm. It is usually suggested that the latter changes are brought about by exposure but there is no convincing proof for the theory. The incidence of the changes increases with chronological age after 60, no matter what the previous occupation or mode of life of the individual, and they are equally prevalent both in the female and in the male. Histological examination of the affected areas reveals the presence of an increase of elastic staining material. Unna (1896) described changes in the staining properties of both collagen and elastin and clearly recognized that metachromatic staining was obtained with a variety of tissues. It is surprising, therefore, that later workers persisted in referring to the dermal changes in senile elastosis as indicating an excess of elastic tissue. Tattersall and Seville (1950) working in my Department, in co-operation with Astbury and Reed (Tunbridge *et al.*, 1952), were able to show that the elastic staining material was altered collagen. Other workers, notably Gillman and co-workers (1954), have since confirmed and

extended our findings. The fact that the incidence of senile elastosis increases with chronological age strongly suggested that the reaction of one of the constituents of the dermis varied with age. Keech (1955) demonstrated a different degree of reactivity of collagen, from the skin of the abdominal wall, to collagenase in different age groups. Burton and co-workers (1955) extended the studies and found that the reaction of skin collagen to other enzymes and alkaline buffer solutions also varied with the age of the subject from which the sample of dermal collagen was obtained. We claim that these findings indicate that definite changes occur in the composition and the reactivity of human dermal collagen with chronological age but as yet we cannot exclude the possibility that these changes are brought about as a result of environmental factors or disease.

The marked degenerative changes that occur in the aorta and major arteries of the elderly have long attracted scientists to the study of physical properties of these structures. The loss of elasticity is all too apparent, amounting in the case of the Mönckeberg type of sclerosis to complete rigidity of the vessel, the calcification in the latter disorder being confined mainly to the media. Lansing, Rosenthal and Alex (1950) estimated the calcium content of the aorta and pulmonary artery in different age groups, always selecting a non-atheromatous area for analysis. They observed a striking increase in the calcium content of the aorta with increasing years but only a slight increase in the pulmonary artery. Further, Lansing and co-workers (1951), using chemical and chromatographic analyses found that the amount of elastic tissue in the aorta after the second decade was constant, or might even increase slightly. The amino-acid analyses, however, did reveal differences in the composition of the aorta from the young and from the elderly. There were significant increases in the amount of aspartic and glutamic acids in the elderly.

The discovery by Baló and Banga (1949*b*) of the enzyme elastase in pancreatic tissue provided a new approach to the study of arterial changes. The precise nature of elastase is

still not known, but the purest preparations so far prepared contain at least two enzymes, one of which is proteolytic. Further, Hall (1955) has shown that a metal is concerned in the reaction between elastin and elastase and that the metallic ion is calcium. The concentration of the calcium ion is important and can determine the form of the reaction. Baló and Banga (1949*a*) suggested that elastase was normally present in the body but prevented from acting in the normal individual due to the presence of an inhibitor. Graham and Saxl (1957) have been able to confirm the presence of an inhibitor in normal serum and have suggested that its action may be determined by the state of the tissue with which it is in contact. Thus, physical stress might provoke changes in the aorta and in consequence the altered tissue might take up the inhibitor substances releasing elastase and so exacerbating still further the degradation. This process is also accompanied by the liberation of fatty material and so other well recognized features of arterial degeneration could be explained. Reference has already been made to the work of Lansing and his finding that the amino-acid composition of elastin from the aorta varies with age. We have found that the susceptibility of the aorta to the action of elastase varies with age. The aorta, however, is a very complicated structure. Morphologically at the level of the light microscope at least two distinct forms of elastic tissue can readily be observed and both are intimately associated with collagen fibres. It is, in fact, extremely difficult to separate the collagen from the elastic material. The majority of the chemical methods used to free elastic tissue of collagen are drastic and undoubtedly bring about some degradation of the material. It is thus very interesting that elastase should show a greater degree of activity with older specimens of aorta but such changes are not necessarily a direct result of ageing processes but could well be brought about by the effect of disease. Using elastase we have further been able to show that there are chemical differences between the elastic material as derived from the aorta and from the ligamentum nuchae. Wood (1955), examining the extensile

properties of collagen from different sites, has also been able to show that the collagen from ligamentum nuchae has greater extensile properties than collagen from other sites.

These observations indicate the difficulties in interpretation of experimental findings. Firstly, the results obtained with detached tissue do not necessarily represent what would occur in the body in life. Secondly, it is impossible to know how far the changes are the result of disease processes and how far they can be taken as evidence of a biological process of ageing.

The obvious limitations of human sources of material, whether the whole organism or an organ, must not blind us to the constant stimulus afforded by the study of such material. The changing age composition of the population in what is so frequently referred to as Western society is focussing everyone's attention upon the present limitations of the aged. We are all human and therefore the problem is personal, perhaps too personal and not approached in the detached way that it should be. The clinician may incorrectly attribute changes to a process of ageing but his observations that apparently similar stresses may produce different effects in different age groups, that the skin of the face changes with chronological age, but to a greater extent than the skin of the back, that generalized atheroma is considered almost a normal finding in the aortic arch, for a person over the age of 60, yet is rarely found in the pulmonary artery of the same individual, open up lines of thought which stimulate the investigator and lead us to hope that one day we shall be able to decide whether the so-called "effects of age" are merely the consequence of accumulated trauma to the organ or to the individual or that there is really an alteration of structure and of function associated with chronological age.

It is my hunch that we are more likely to obtain a key, possibly the key, to the problem "What is ageing?", or "Is there a biological process of ageing?", from colony studies such as those of Dr. Mühlbock or from the researches of Dr. Parkes than from human studies. Of course, what applies to the mouse or to the hamster need not apply to the human.

The methods in routine use in clinical medicine today would seem unlikely to contribute much to our understanding of the problem of ageing. Nevertheless, human studies are worth while even if they only assist us to understand the processes which appear to accompany chronological age and so enable us to make a contribution to the practical problems of an ageing population or what has been aptly referred to as "adding life to years".

REFERENCES

Baló, J., and Banga, I. (1949*a*). *Nature, Lond.*, **164**, 491.
Baló, J., and Banga, I. (1949*b*). *Schweiz. Z. Path.*, **12**, 350.
Baló, J., and Banga, I. (1950). *Biochem. J.*, **46**, 384.
Burton, D., Hall, D. A., Keech, M. K., Reed, R., Saxl, H., Tunbridge, R. E., and Wood, M. J. (1955). *Nature, Lond.*, **176**, 966.
Cameron, G. R. (1955). Ciba Foundation Colloquia on Ageing, **1**, 16. London: J. & A. Churchill Ltd.
Gillman, T., Penn, J., Bronks, D., and Roux, M. (1954). *Nature, Lond.*, **174**, 789.
Graham, I., and Saxl, H. (1957). In press.
Hall, D. A. (1955). Old Age in the Modern World, p. 165. Edinburgh: Livingstone.
Hobson, W., and Pemberton, J. (1955). The Health of the Elderly at Home. London: Butterworth.
Howell, T. H., and Piggott, A. P. (1949). *Geriatrics*, **4**, 281.
Howell, T. H., and Piggott, A. P. (1950). *Geriatrics*, **5**, 90.
Howell, T. H., and Piggott, A. P. (1951). *Geriatrics*, **6**, 85.
Howell, T. H., and Piggott, A. P. (1952). *Geriatrics*, **7**, 137.
Howell, T. H., and Piggott, A. P. (1953). *Geriatrics*, **8**, 215, 267.
Keech, M. K. (1955). *Ann. Rheum. Dis.*, **14**, 19.
Lansing, A. I., Rosenthal, T. B., and Alex, M. (1950). *J. Geront.*, **5**, 112.
Lansing, A. I., Roberts, E., Ramasarma, G. B., Rosenthal, T. B., and Alex, M. (1951). *Proc. Soc. exp. Biol., N.Y.*, **76**, 714.
Sheldon, J. H. (1948). The Social Medicine of Old Age. Oxford University Press for The Nuffield Foundation.
Spence, J., Walton, W. S., Miller, F. J. W., and Court, D. M. (1954). A Thousand Families. Oxford University Press.
Tattersall, R. N., and Seville, R. (1950). *Quart. J. Med.*, **19**, 151.
Tunbridge, R. E., Tattersall, R. N., Hall, D. A., Astbury, W. T., and Reed, R. (1952). *Clin. Sci.*, **11**, 315.
Unna, P. G. (1896). Histopathology of the Diseases of the Skin. Translated by N. Walker. New York: MacMillan.
Vischer, A. L., and Roulet, F. C. (1952). *Virchows Arch.*, **321**, 652.
Wood, G. C. (1955). Old Age in the Modern World, p. 157. Edinburgh: Livingstone.
Zonneveld, R. J. v. (1955). Old Age in the Modern World, p. 349. Edinburgh; Livingstone.

DISCUSSION

Best: Prof. Tunbridge has already referred to some of the work of Dr. Banga, and I would like to invite you, Prof. Baló, to give us a summary of your findings; perhaps a statement of what you think about the present position of elastase.

Baló: I reported on elastase in 1948, at the First Congress of Italian Pathologists, at Forli. I had worked on this problem since 1938, and at first I followed the work of Anitchkow. He found that after the administration of cholesterol one can bring about a special type of alteration of the arteries, called "cholesterol atherosclerosis", where cholesterol is deposited in the arteries; but it is not always clearly distinguished from other types of arteriosclerosis. In April 1956 I visited Prof. Anitchkow in the Soviet Union and discussed this problem with him; he believes that this is the only type of arteriosclerosis and calls it "atherosclerosis". I do not agree with this, but agree with Prof. Kallmann who has distinguished between this type of cholesterolsclerosis and the destruction of elastic fibres which is, in fact, different from atherosclerosis.

My work was carried out in collaboration with Dr. Banga and we have published our findings (1949, *Schweiz. Z. allg. Path.*, **12**, 350; 1950, *Biochem. J.*, **46**, 384). My first method was to put sections from the carotid artery, which is rich in elastic fibres, into a solution of pancreatic extract to give a watery extract; later I took degreased specimens of the pancreas and these again gave watery extracts, and I found when putting the histological sections in the solution that the elastic fibres of the arteries dissolved.

Later on, biochemical methods were introduced and finally we carried out the experiment in both ways. Because the test with the sections of elastic arteries in elastase is very accurate, both methods can be used to determine the elastolytic activity of a solution.

We have considered that trypsin (which is a general proteolytic enzyme in the pancreas) or chymotrypsin or some other enzymes might be responsible for this solution of elastic fibres, but we found that crystallized trypsin, which we received from Prof. Northrop, does not dissolve the elastic fibres whereas elastase dissolves them readily. We purified our specimens, and Dr. Banga obtained crystalline elastase.

We were, of course, interested in the problem of how elastase worked in the human organism. Early in our work we found that both human and animal serum contain an inhibitor (to which Prof. Tunbridge has referred) and that very small quantities of serum can inhibit the action of elastase. As a result of these findings, we tried to define the possible rôle of elastase in the organism. We removed the pancreas of the dog, and on doing this we found some destruction of the elastic fibres. These dogs were kept alive, some for more than one year and some for less than one year, for 7, 10 or 12 months, which I believe is sufficient to demonstrate the effect of the lack of elastase. In all these experiments we found some alterations in the arteries after removal of the pancreas. In some of these animals which I investigated I was at first led to believe that remarkable alterations had occurred in the arteries; then I began to

have doubts. Finally I discovered that this was spirocercosis of the dog, which is a very common disease in our country. In many cases, I found these parasites which produce many interesting changes, especially in the aorta and large arteries of the dog. I have since proved that in spirocercosis the parasite itself produces elastolytic enzyme, and it is interesting to note that whenever the parasite is found in the wall of the aorta the elastic fibres are all dissolved and some of the dogs die from rupture of the aorta.

The next step was to try to find out what the importance of this finding is in the human being. I wanted to know whether elastase is present in the pancreas of healthy individuals, and in what quantities. I have had the opportunity to investigate the pancreas from people who died as a result of an accident, and using fresh material received from the Medico-Legal Institute, I proved that if healthy individuals died as a result of an accident, great quantities of elastase were present in their pancreas. I compared this finding with the findings in diseased individuals, especially the findings in adults, and found that the quantity of elastase decreases with age, so that in old age—especially at the age of about 60—scarcely any elastase is seen in the pancreas.

Arteriosclerosis may be correlated with the disappearance of elastase from the pancreas. Elastase is of decisive significance not only in the decomposition of elastic fibres but also in their synthesis, and is essential to the maintenance of intact elastic fibres. Lack of elastase leads to the degeneration and disappearance of the elastic fibres.

At first we found that the elastic fibres of young and old individuals are dissolved in a different manner, whereas those in the arteries of new-born children are not dissolved, even after three days; so if we take histological sections from the aorta of the newborn or very young child, and put them in elastase solution, the elastic fibres are not dissolved and after three days only slight solution can be observed. On the contrary, if we take sections of the aorta or elastic arteries of an old individual, then the elastic fibres are readily dissolved. I have found that in individuals aged over 60 years the elastic fibres are dissolved in twenty minutes, and this proves that there are differences in the structure of the elastic fibres in young and in old individuals.

Although we continued to work along these lines, no one in our circle of research believed this finding and, therefore, it was of great help to us when Prof. Tunbridge's school in Leeds took up the problem and it was a great pleasure for us when Prof. Tunbridge and his collaborators, especially Hall and Reed, were able to prove that elastase works in this way. Since then, Prof. Tunbridge and his school have carried out important investigations along this line in that they have combined this line of research with electron microscopic investigation of dissolved elastic fibres, and they have made very important contributions to the data on structure of elastic fibres.

In the United States, Lansing and co-workers have taken up this study of the structure of elastic fibres, and a similar study was begun by Schwarz and Dettmer in Berlin and by Findlay, Pepler and Brandt in South Africa. During these studies, the question was asked "Are the

elastic fibres homogeneous or are there different substances in the fibres?" It was believed that there are fibres in the elastic substance but that there is also another cementing material which probably is different from the elastic fibres. Schwarz' and Dettmer's theory was that the elastic fibres are built up of collagen fibres and cementing substance, and these workers found that the fibres which are obtained after dissolution of the elastic fibres have cross-striation like those of the collagen fibres. Recently it was decided that the entire substance reacts to elastase: the cementing substance as well as the fibres are dissolved by elastase, and I believe that these fibres which have a cross-striation corresponding to that of collagen are probably mixed with the elastic substance as in the cervical ligament. In the vessels, elastic fibres and collagen fibres occur together.

Recently, Hall, Keech, Reed, Saxl, Tunbridge and Wood stated that collagen can be transformed into elastin. In collaboration with Banga and Szabó I found that during thermal or chemical contraction-relaxation, a mucopolysaccharide and a soluble protein are dissolved from the collagen fibre. We called the substance left behind after the dissolution of these two substances "metacollagen". We believe that metacollagen is similar to elastin.

Best: That was very good of you, Prof. Baló, at such very short notice.

NUTRITION, LIVER DISEASE AND SOME ASPECTS OF AGEING IN AFRICANS*

T. Gillman

Faculty of Medicine, University of Natal, Durban

Although what I have to say has not been previously prepared as a formal paper, I should nevertheless like to indicate the kind of problems which we meet in the Union of South Africa, and which may have implications for the study of ageing—especially in backward countries. I would also like to outline some of the methods and hypotheses which we are applying in our attempts to understand this material. Before leaving South Africa to come abroad, I prepared a series of coloured lantern slides to illustrate our material, and I am grateful to our chairman for this opportunity of presenting them to you.

Part of the work—and especially that being done in collaboration with Drs. M. Hathorn and N. Lamont—is on the rôle of malnutrition in the production of the liver and cardiac diseases so commonly encountered in the African in Durban. This work is being integrated with our experimental studies on various aspects of the repair of injury in relation to carcinogenesis and ageing. We think that the repair of the continuously inflicted injuries, consequent on chronic malnutrition, may be important in causing the peculiar incidence of various forms of hepatic and vascular disease, primary liver cancers and premature ageing in the African in the Union. My brother and I have previously presented many aspects of this problem in some detail (Gillman and Gillman, 1951).

I feel, at the outset, that it is important to make what

* Since these data, which were presented during the discussion following the papers by Dr. Landowne and Prof. Tunbridge, constitute a full paper, they are being published here in that form.—Eds.

might sound a rather categorical statement, but one which I think is nevertheless near the truth—namely that the entire biology of backward peoples in Africa, the Middle and Far East, and elsewhere in this world, differs profoundly from that of Western civilized people, not primarily because of genetic differences (which cannot easily be assessed), but rather by virtue of a host of environmental factors.

It became clear, some years ago, to my brother and me that many of the diseases in Africans, and the different disease incidence in these people compared with that in South African Europeans, could better be understood if the reactions encountered are viewed as deviations in the "life track", or better, "life pattern" of the African, resulting from chronic malnutrition originating in infancy if not at the time of conception. The concept of acute nutritional deficiencies as the cause of many human diseases, including pellagra, could not adequately explain either the acute nutritional failures themselves or many of the other lesions which we see. In particular, the concept of acute nutritional deficiency does not provide a basis for explaining what we have previously described as "premature ageing", so common among the Africans.

At that time, I was working with my brother, Prof. Joseph Gillman, in Johannesburg. Since then I have moved down to Durban. In view of the fact that the population of Johannesburg, particularly the African population, is rather varied—being a highly industrialized city with a big influx of migrant labour (largely for work in the mines)—it became of interest to study also the situation in Durban, Natal, in which the population is somewhat more uniform in composition, and somewhat more stable. What I have to say now is confined entirely to the African negro.

The *estimated* life expectancy of the African (there are no vital statistics available in our country for Africans) is extremely low—somewhere between 40 and 45 years. Associated with this low life expectancy we have found many indications of premature ageing and a whole series of reactions which differ from those usually seen among Europeans in

South Africa and elsewhere. For example, the incidence of carcinoma is considerably lower in the African; but, when carcinoma does occur, as Dr. Charles Berman has shown so clearly (1951), about 90 per cent of cancers in African males are cancers arising primarily from hepatic epithelium. But this is probably already well known to many of you. I think that Dr. Mühlbock's remarks may have some significance for the understanding of this peculiar carcinoma incidence. Dr. Mühlbock observed that many of his mice that have apparently a lethal gene, and die early, do not get carcinoma. This low, but peculiar, carcinoma incidence in the African male may be due to several things. One may be the fact that the African just does not seem to live long enough to develop the other types of cancers so common among Europeans. After all, certain biological reactions, once initiated, seem to require fairly fixed times for their completion, e.g. the action of insulin or of oestrogen in certain specified physiological circumstances, as well as the time required for the production of certain types of carcinomas, such as the lung cancers which we have found to follow the application of methylcholanthrene to the skin (Gillman, Hathorn and Penn, 1956). Perhaps this biological peculiarity may, in part, explain the low incidence of non-hepatic cancers in Africans.

However, there may perhaps be other reasons for this peculiar cancer incidence in the African, and we have tried to find something in the African which would account for this peculiar cancer incidence. In so doing we came across a number of other unusual reactions which we feel have a common basis. Among them is the fact that the African liver is severely injured, with great frequency and on repeated occasions, virtually from birth. The nature of the hepatic reactions in Africans, as my brother and I have already described (Gillman and Gillman, 1948, 1951), varies at different ages.

Although I was accustomed to seeing a considerable amount of liver disease in Johannesburg, I have been shocked by the apparently even higher incidence in Durban Africans. In a

recently initiated study* we did 82 liver puncture biopsies [by a trans-abdominal method previously described (Gillman,

Histological Findings	*No. of Cases*	%
Cirrhosis *without* siderosis	5	6
Cirrhosis *with* siderosis	33	40
Siderosis *without* cirrhosis	32	39
Primary carcinoma	3	4
Secondary carcinoma	1	1
Minor changes ("normal")	8	10
TOTAL	82	100

RELATION OF SIDEROSIS TO CIRRHOSIS

With Cirrhosis	*No.*	%
Without siderosis	5	12
With siderosis	33	88
TOTAL	38	100

With Siderosis	*No.*	%
With cirrhosis	33	51
Without cirrhosis	32	49
TOTAL	65	100

FIG. 1. Microscopic findings in 82 needle biopsies of the liver.

Gillman and Bryden, 1945)] on patients who presented in the ward with clinically recognizable hepatic enlargements. This,

* We would like here to record our thanks to Drs. N. R. Pooler, F. J. Davidson and J. K. Drummond for so kindly making available their clinical material.

then, was a selected population of people that we biopsied in one ward in the hospital over a period of only two months. In these biopsies, the incidence of cirrhosis, defined on the most stringent histological criteria, was 46 per cent. Severe siderosis occurred in 88 per cent of these cirrhotics, only 12 per cent being non-siderotic cirrhosis (Fig. 1). We did not diagnose cirrhosis on the basis of the deposition of moderate amount of fibrous tissue in the portal tracts, i.e. portal fibrosis, but only when gross architectural distortion of the liver was detected in association with such fibrous tissue accumulation such as I will show in a moment. These siderotic livers had iron contents which were found, in previous studies (Gillman, Mandelstam and Gillman, 1945), to range from 0·2 to as high as 5·0 per cent of the dry weight of the liver. Primary carcinoma of the liver occurred in 4·0 per cent, i.e. in three cases. Secondary tumours are rarely seen in the African liver, and we found only one such case. We classified as relatively normal only 10 per cent of the livers seen in these cases. This is more or less the inverse of what one finds in Europeans, in whom the incidence of cirrhosis, varying in different populations, ranges from 1·5 to 4·2 per cent.

Apart from reporting this high incidence of severe liver disease, I would like to indicate the kind of reactions which we found associated with it. We have now got to the stage where, having correlated clinical and histopathological examinations and laboratory investigations in one series of cases, we can now diagnose this hepatic siderosis with reasonable precision, solely on clinical criteria.

Fig. 2 typifies the kind of patient with whom we have constantly to deal. The distended abdomen, due to ascites, is frequently associated with palpable hepatic enlargement. Gynaecomastia, varying from nipple enlargement (Fig. 3) to frank enlargement of the entire breast (Fig. 4) is common and is often associated with axillary and also with pubic alopecia (Fig. 5). In Fig. 4 the enlargement of the breasts is more obvious, both the areola and nipple being enlarged, while the underlying breast tissue was also palpably hyperplastic. The

labial stomatitis, a stigma of persistent low-grade malnutrition, so frequent in these cases, is also apparent in Fig. 4.

Another clinical feature which we encountered, and which, I have felt for many years, has been misdiagnosed in the African, is the so-called "arcus senilis". Fig. 6 shows what in the African I would call (and consider comparable with similar ocular changes in Europeans) a "true" arcus senilis. However, the limbic change shown in Fig. 7, and which is frequently, in my opinion, misdiagnosed as arcus senilis, I do not consider to be a "true" arcus senilis, but the brownish colour here is probably a deposition of iron pigment in the limbic portion of the cornea and associated with a marked pigmentation of the exposed conjunctiva. The entire picture in the African seems indistinguishable, clinically and histopathologically, from what is generally regarded among European people as haemochromatosis. We refer to this condition in the African as "nutritional siderosis".

The hepatic siderosis presents itself in several forms representing, we think, different stages in the progress of this disease. Most of the slides that follow are coloured photomicrographs of sections from liver biopsy material stained to demonstrate iron. Thus, Fig. 8 represents the appearance of the mild form of hepatic siderosis. The iron, at this stage of the disease, is encountered mainly in the liver cells themselves, usually confined to the biliary poles of the cells (Fig. 9), with small amounts only in occasional phagocytes, either in the sinusoids or in the portal tracts. This probably represents a very early stage in this disease, and as stated elsewhere (Gillman and Gillman, 1951), we believe the iron to arise initially as a result of disturbances in the metabolism of intracellular iron-containing enzymes. Hence the name "cytosiderosis".

In what probably represents a slightly more advanced lesion the iron is now aggregated in clumps within the liver cells and seems also to be accumulating rapidly, although probably intermittently and in graded quantities, in the Kupffer cells, or in clumps of phagocytes in the lobules as well

as in the portal tracts (Fig. 10). During an even later stage in this disease, when the accumulation of iron in liver cells may diminish temporarily, the amount of siderin within the liver cells themselves may be relatively small while quite considerable amounts may be found in the Kupffer cells and in the portal phagocytes. Now, this kind of picture seems to have led people to believe that we are dealing with some form of primary reticuloendothelial siderosis, whereas my brother and I have maintained (and all the studies which we have conducted more recently in Durban confirm the original contention) that this seems to be primarily an intracellular lesion and only secondarily a reticuloendothelial one. We still maintain this view despite the opinions of some South African workers (Higginson, Gerritsen and Walker, 1953). It is apparent from Fig. 10 that iron accumulates in massive clumps in the portal tracts, and even the collagen in the associated fibrous tissue may take up iron.

A serial section of this same liver, but stained for connective tissue, revealed the considerable amount of connective tissue present although it may be partially obscured by the large amounts of portal iron shown in the previous slide. In such sections iron-containing pigment appears as coarse brown granules. However, it is misleading to attempt to assess the

PLATE I

FIG. 2. Characteristic clinical picture of advanced nutritional siderosis with cirrhosis in an African. Note gross ascites, loss of hair of outer third of eyebrows, skin changes and emaciation.

FIG. 3. One form of gynaecomastia producing elongated, "top hat" type of nipple enlargement.

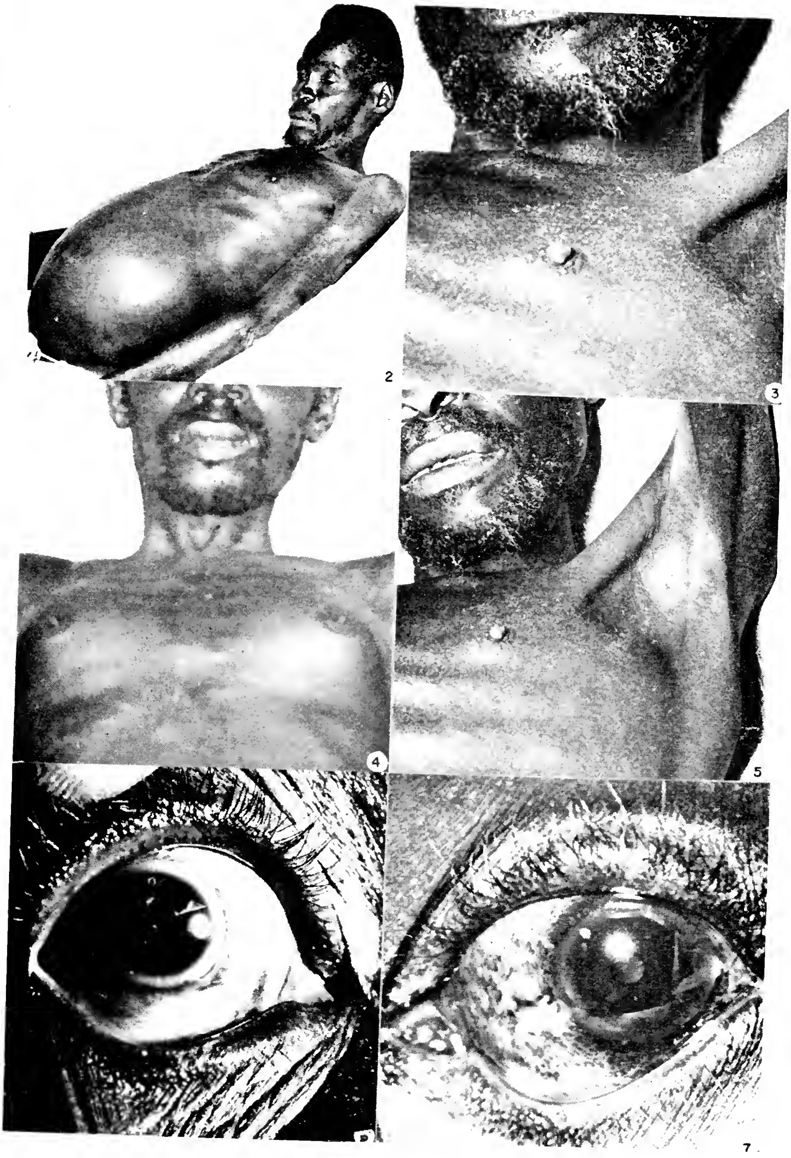
FIG. 4. Grosser degree of gynaecomastia with nipple and areolar enlargement, as well as hyperplasia of breast tissue. Note labial and angular stomatitis.

FIG. 5. Axillary alopecia, frequently observed in advanced nutritional siderosis with cirrhosis.

FIG. 6. Classical European type *arcus senilis* in an African, with opaque, pearly-white limbic ring and minimal conjunctival pigmentation.

FIG. 7. Golden brown transparent limbic ring (medial part of limbus), gross conjunctival pigmentation and Bitot-like spot medial to cornea. The limbic ring resembles the classical Keiser-Fleischer ring of hepatolenticular degeneration.

PLATE I



[*facing page* 110

PLATE II

Fig. 8. Severe cyto-siderosis with iron pigment confined almost entirely to hepatic epithelium. Minimal Kupffer cell siderosis (type 2 siderosis). × 164.

Fig. 9. Higher power view of type 2 siderosis—outlining of bile capillaries by intra-hepatocellular iron pigment. × 820.

Fig. 10. Moderately severe type 3 siderosis with lesser amounts iron pigment in liver cells but considerable amounts in Kupffer cells and phagocytes in the thickened portal tracts. × 82.

Fig. 11. Appearances of liver biopsy from patient with gross nutritional siderosis and cirrhosis. Marked siderosis of liver cells, Kupffer cells and phagocytes in dense bands of fibrous tissue. × 82.

PLATE II

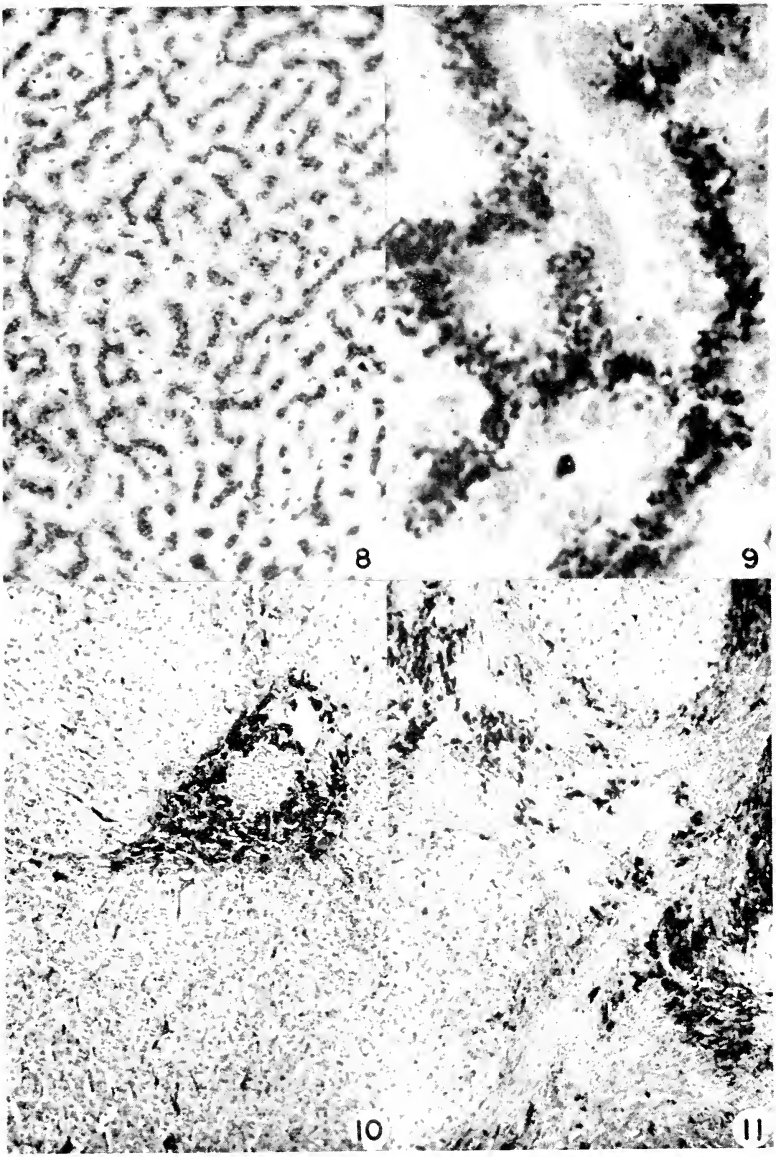
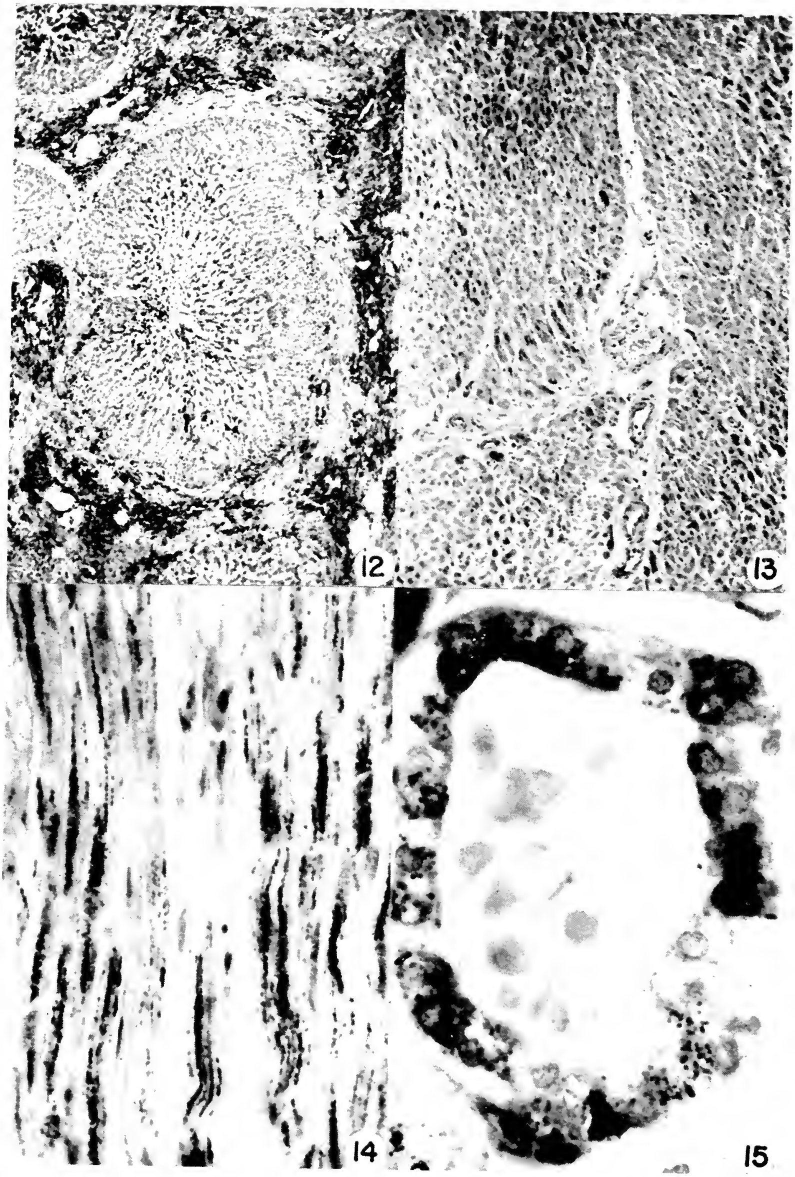


PLATE III



amount of iron in a specimen from a siderotic liver, if sections have not been properly stained, specifically for iron.

Severe simultaneous siderosis of both hepatic epithelium and reticuloendothelial cells, associated with cirrhosis, is exemplified in Fig. 11. It seems that, at this stage of the disease, three processes occur simultaneously, or at least in rapid succession—namely, (1) the progressive accumulation of iron in the liver cells associated with (2) the continuous movements of large quantities of iron, from the diet and possibly from dying liver cells into the Kupffer cells and the portal tracts, the latter apparently reacting with massive fibrosis culminating in (3) gross architectural distortion, or cirrhosis. The histological picture established in biopsy specimens in this case was subsequently confirmed at postmortem (Fig. 12). Interestingly enough, on admission to hospital the patient in this instance, as in many others, was not initially diagnosed as suffering from hepatic disease, but was considered to be in congestive cardiac failure with an associated ascites.

We have, for some time, suspected that congestive cardiac failure, very common in the relatively young African, and often of unknown aetiology, may be due to a basic derangement in the metabolism of the intramyocardial iron-containing enzymes, similar to that in the liver. This intracellular metabolic lesion may perhaps underlie the cardiac failure, disturbed electrocardiograms and the accumulation of iron within the myocardial cells themselves—a possibility which

PLATE III

FIG. 12. Low power view of section from same liver as Fig. 11, but obtained at postmortem. Gross siderosis (type 2 and type 3) with severe cirrhosis superimposed, confirming liver biopsy diagnosis. × 36.

FIG. 13. Low power view of cardiac muscle from same case whose liver is shown in Figs. 11 and 12. Note patchy intra-myocardial siderosis with absence of iron from connective tissue. × 82.

FIG. 14. High power view of same myocardium as Fig. 13, showing considerable accumulations of iron pigment, in fine granules and clumps, within myocardial cells; iron absent from connective tissue. × 820.

FIG. 15. High power view of thyroid follicle from same case as Figs. 11–14, showing granules and coarse clumps of intra-epithelial iron pigment, which is absent from peri-follicular connective tissue. × 720.

we are presently investigating. Examination of cardiac muscle in this, and in several other cases, thus far available, revealed an extensive, albeit patchy, deposition of iron pigment within the myocardial cells (Figs. 13 and 14) as well as in the liver. It is known from Sheldon's work (1935) that cardiac failure may supervene in relatively young European haemochromatotics. However, at this stage, we still prefer to call this condition in the African nutritional siderosis, with or without cirrhosis.

There is quite strong evidence to support our view that the siderosis of the liver and other organs is due initially to the liberation of iron within parenchymal cells from their iron-containing enzymes. Thus, at the outset iron is encountered essentially within the hepatic epithelium rather than, as occurs in haemolytic states, mainly in the reticuloendothelial cells. In the later stages of the disease iron again occurs primarily within parenchymal cells such as the heart (Figs. 13 and 14), the thyroid epithelium (Fig. 15), etc., in which organs very little iron is observed in the connective tissues even when the parenchymal cells are markedly siderotic. Whatever the underlying cause and pathogenesis of this type of siderosis, in the ultimate analysis, the excessive iron in all the tissues must be derived from the diet. It is our belief that the failure of the normally present alimentary blockade to iron absorption is secondary to some nutritionally based derangement in the iron-containing enzymes within the parenchymal cells in many organs and tissues. The reticulo-endothelial siderosis we consider to occur late in the disease and to follow the liberation of iron consequent on the death and disintegration of iron-laden parenchymal cells.

Associated with these hepatic lesions we have consistently encountered profound changes in the plasma proteins. The total proteins may be more or less normal but one of the striking and consistent findings in these patients is a profound drop in the plasma albumin associated with a marked increase in the plasma globulin with a consequent complete reversal of the albumin: globulin ratios. These changes, like the siderosis,

are not reversible by any of the therapeutic measures hitherto tried by us over many months and even years. This indicates the persistence of profound metabolic disturbances after resolution of the acute breakdown—whatever form the latter may take.

One of the hypotheses underlying our work in Durban is that the frequency of primary liver cancer in the African may perhaps be due, in large measure, to the virtually continuous repair of the chronic hepatic lesions which are almost invariably present in these people. Hence our basic interest in wound healing and its relations to neoplasia, experimentally and in man.

In the light of our findings, both in Johannesburg and in Durban, it seems justifiable to question the opinions today being expressed about the incidence and aetiology of various diseases, especially in Africa. On the basis of ethnological comparisons of the incidence of various types of diseases and their associated biochemical changes, conclusions are often drawn about aetiology or pathogenesis. In particular, I find it difficult to accept the interpretations given to the correlation between alterations in blood lipids and the incidence of coronary disease in Africans, Coloureds and Europeans in the Union—especially when attention is not simultaneously paid to the associated underlying pathological processes common among these peoples. We have shown that the incidence of hepatic cirrhosis in Europeans in South Africa is in the neighbourhood of 3–4 per cent of autopsy cases; in this racial group coronary disease is quite frequent, and may or may not be correlated with a high intake of fat or associated alterations in the blood lipids. In another racial group—the Africans—the incidence of coronary disease seems to be low and this has been attributed to the low fat intake and an associated low blood lipid. However, hepatic cirrhosis, as I have shown, is extremely common in the African. To attempt, in such circumstances, to correlate directly, either the intake of fat or the blood lipid level with the incidence of coronary disease in these two races may be misleading if attention is not simultaneously

devoted to the underlying severe liver disease and its associated metabolic repercussions. And I think the same criticism probably applies to similar studies emanating from backward countries, in which only a limited number of parameters have been measured.

I hope by giving you some idea of the kind of material with which we deal in South Africa, that I have provided an outline of some aspects of the problems of ageing as *we* see them in relatively backward populations. It seems that the kind of problems we encounter differ in many respects from those that you describe among relatively well fed European populations. On the other hand, since this is a conference on the methodology of ageing, I also hoped to indicate the need for defining, with greater precision, the kind of investigations which are required if, as I think they should be, the problems of ageing are to be dealt with in terms of the total life patterns of peoples differing profoundly in their nutritional states, from birth to death, and whose physical environments also differ so markedly as a result of climatic and socio-economic factors.

REFERENCES

BERMAN, C. (1951). Primary Carcinoma of the Liver. London: Lewis.
GILLMAN, J., and GILLMAN, T. (1948). *Lancet*, **i**, 169.
GILLMAN, J., and GILLMAN, T. (1951). Perspectives in Human Malnutrition. New York: Grune & Stratton, Inc.
GILLMAN, J., GILLMAN, T., and BRYDEN, J. G. (1945). *S. Afr. J. med. Sci.*, **10**, 53.
GILLMAN, J., MANDELSTAM, J., and GILLMAN, T. (1945). *S. Afr. J. med. Sci.*, **10**, 109.
GILLMAN, T., HATHORN, M., and PENN, J. (1956). *Brit. J. Cancer*, **10**, 394.
HIGGINSON, J., GERRITSEN, T., and WALKER, A. R. P. (1953). *Amer. J. Path.*, **29**, 779.
SHELDON, J. H. (1935). Haemochromatosis. Oxford University Press.

THE USE OF INBRED STRAINS OF ANIMALS IN EXPERIMENTAL GERONTOLOGY

O. MÜHLBOCK

The Netherlands Cancer Institute, Amsterdam, Holland

Introduction

IN gerontology, as in other fields, animal experiments offer the advantage that certain factors can be kept constant, whereas other factors can be altered as required. Another advantage lies in the fact that animal experiments can be repeated as often as necessary until unmistakable results have been obtained. The difficulty lies in the interpretation of results obtained in animals and in the correlation with symptoms observed in human subjects. It can be presumed that animals do not differ from man in the basic principles of the process of ageing, so that valid conclusions can be reached. The choice of the experimental animal is dependent on the problem to be investigated. For purely practical and economic reasons, however, small rodents such as mice and rats are usually employed for gerontological investigations.

As in other fields of scientific experimental investigation, it has been apparent from the outset that the genetic constitution of the animal is of paramount importance in the study of senile changes. As in all other biological investigations, moreover, considerable individual variations in the response to environmental influences must be taken into account. In view of these two facts the availability of inbred strains is of great importance.

Development and Maintenance of Inbred Strains

Inbred strains are usually obtained by brother-to-sister matings until all individuals are genetically identical and

their genetic constitution can change only by mutation (Russell, 1941). In mice, it requires at least 20 generations of inbreeding before we can speak of an inbred strain. If a strain is to be maintained with maximum homozygosis, there should be no relaxation of inbreeding. It should be recognized that the strain may break up into many separate lines unless the choice of the breeding pairs is made with reference to a pedigree chart. It is essential that only one central trunk of the inbreeding tree be maintained. Because of mutations, brother-to-sister matings alone, i.e. without limitation to the central trunk, can produce a multitude of sub-lines all of which may differ (Heston, 1945).

The criteria used in proving that all individuals of a pure strain are genetically identical should first be considered. It need not be said that the general aspect and the morphology of the organs should be identical. Another requirement to be met is that neoplasms developing in an animal of a certain strain should be transplantable to another individual of that strain. The same holds true for tissues and organs which, following transplantation to another individual, continue to grow and to function as they did in the original animal.

The ovaries are known to be transplantable even in incompletely homozygous strains; the skin, however, requires a considerably more marked genetic similarity, as indicated by the experiments of Billingham and Parkes (1955). The most exact criterion used in our laboratory, however, has been found to be a procedure known as parabiosis, i.e. the union of two animals by side-to-side suturing, not only of the skin but also of the thoracic and abdominal muscles and, for fixation, of the shoulders and the iliac bones. Communication of the two circulations is established within a few days. A satisfactory parabiosis with normal lifespan of both partners, however, is established only in completely genetically identical animals.

Besides the accurate administration of the inbred strains, the care and hygiene of the animals require maximal attention. It is desirable that the environmental factors be kept as

constant as possible. The systems used to ensure this are dependent on the available resources, geographical situation, etc. Prevention of infections is essential in prolonged experiments such as are required for gerontological investigations.

At the Amsterdam Cancer Institute the animals are kept in cages made completely or partly of glass, which enables direct observation to be made at all times.

Special attention has been given in recent years to the number of animals kept together in one cage. The starting point of a series of investigations was the observation that in cages containing a larger number of animals the incidence of mammary carcinoma was clearly lower than that in the cages containing a smaller number (Mühlbock, 1950, 1951). Investigations subsequently made confirmed this observation. The percentage of mammary carcinoma was highest among the animals kept separately in one-animal cages. Since the other conditions were exactly identical, it is as yet impossible to provide a satisfactory explanation of this phenomenon.

That psychic factors can be involved is demonstrated by the investigations made at our Institute by van der Lee and Boot (1955). Regular check-ups on the oestrus cycle of female mice repeatedly revealed prolongation of the dioestrus period. Detailed analysis of this phenomenon showed that these dioestrus periods indicate a state of spontaneous pseudopregnancies. A striking feature was found in the fact that the phenomenon was only observed regularly if the cages contained more than one female.

It was demonstrated that these pseudopregnancies were not the result of attempts at copulation among the females. By resection of the olfactory bulbs it was shown that the cause of the pseudopregnancies should be sought in the olfactory sense. These pseudopregnancies, therefore, are caused by stimulation via the central nervous system of the pituitary. This example is given in order to demonstrate that the number of animals kept in a cage is a factor to be taken into account.

In old males, another phenomenon depending on the housing condition is frequently encountered. In some

strains the males begin to neglect themselves with increasing age. As a result they are often infested with lice, which results in marked anaemia. The usual disinfectants have failed, in our experience. At our laboratory an excellent means of controlling infestation with lice was found by keeping old males with young females in one cage. The young females take good care of the old males, as a result of which the latter survive much longer.

As has been discussed by Comfort (1956), the choice of material in experimental gerontology depends on the nature of the problem with which one is dealing. Investigations into the genetic aspects of the ageing process can only be made with inbred strains. For many purposes the F_1 hybrids between two inbred strains may be most suitable. These F_1 hybrids have a uniform genetic constitution. Their chief advantage is the hybrid vigour. They are therefore admirably suited for most experiments in which healthy vigorous animals are required. Moreover, Grüneberg (1954) has recently drawn our attention to the fact that in the case of some multifactorial characters, at least, the homozygous state tends to be less stable developmentally than the heterozygous one. That may be another explanation for the fact that, for a number of purposes, inter-strain F_1 hybrids are to be preferred. The disadvantage of F_1 hybrids is that they will not breed true. The F_2 hybrids have a great genetic variation with the genes provided by the parental strains.

The point is stressed that in spite of an identical genetic constitution and in spite of controlled environmental conditions the variation between individuals may still be considerable. That point is illustrated in the following experiment (Mühlbock, 1950):

Sisters from the same litter in the genetically pure dilute brown strain were invariably kept together in a cage. The conditions of life were consequently completely identical. The time of occurrence of the first mammary carcinoma in a litter was noted. The experiment covered 88 animals from 22 different litters. The sister with the first tumour was made a

starting point for comparison. Mammary cancer subsequently occurred in 74 per cent of the remaining 66 animals. Only 12 sisters (i.e. 18 per cent) also developed a neoplasm within a month. The other animals showed a neoplasm within a period which in extreme cases amounted to over a year.

The Lifespan

The following example shows the survival curves of two strains of our mouse colony and their hybrids. Fig. 1 demonstrates the difference in lifespan of the two strains and the

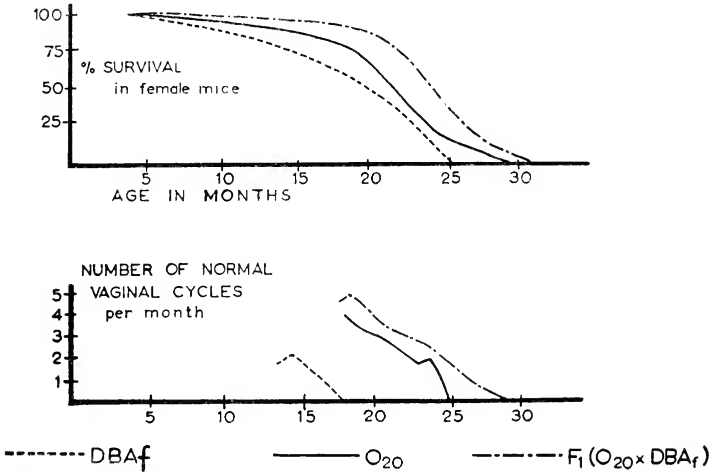


FIG. 1. Survival curves and endocrine ovarian function in ageing inbred mice.

increased lifespan of the hybrids. The heterosis of the F_1 hybrids is also apparent in the physiological activity of their ovaries as expressed in the number of normal oestrus cycles occurring monthly in old age (Thung, Boot and Mühlbock, 1956) (Fig. 1).

The average lifespan of the hybrids is 24 months and the maximum 30 months. In the majority, the end of the period of fertility is reached between 12 and 20 months, at an average

age of 16 months. Vaginal smears showed that cyclic production of oestrogenic hormones is nearly always continued after the termination of the period of fertility, and in many cases until death.

Histological investigation of the ovaries showed that follicles may still be formed in old age, while ovulation is only possible in exceptional cases. However, the ova show signs of reduction both in number and in quality. Transplantation of fertilized ova obtained from young animals into the uterus of old pseudopregnant mice was not followed by pregnancy. This shows that the loss of fertility is not caused exclusively by anomalies in the production of ova (Boot and Mühlbock, 1954).

Causes of Death

Inbred strains will often show a strain-specific pattern of senile disease. The high incidence of such diseases within the strain concerned enables us to study specific causes of death. In females of several strains, which are specially kept in Cancer Institutes, a high incidence of mammary carcinoma as a cause of death is observed. In the well known DBA strain, for instance, the mammary-tumour incidence was 59 per cent in virgin females at an average age of 546 days (Mühlbock *et al.*, 1952). The non-tumour bearing mice died at an average age of 537 days. That means that most of these non-tumour bearing mice died before they developed mammary cancer.

We can easily eliminate the factor which is responsible for the high incidence of mammary cancer in this strain by means of appropriate foster nursing by a mother without the mammary-tumour agent. The incidence of mammary cancer then drops to 4 per cent in virgin females. The lifespan in the females without tumours is prolonged to 598 days as against 546 days. As shown in Table I, 87 per cent of these females die without any gross lesions, besides being very atrophic. In other strains too, the percentage of deaths without easily recognizable cause is of the same order.

One task of experimental gerontology is to analyse more closely the causes of death in these animals. Our attention was first drawn to a form of amyloidosis which was investigated by Thung and co-workers (Thung 1955, 1956; Thung and van Rijssel, 1954).

Generalized infiltration of tissues with an amyloid-like substance is a frequent occurrence in old mice. If localized in the kidneys, this condition may lead to fatal renal disease, as

Table I

CAUSES OF DEATH IN VIRGIN FEMALES OF DIFFERENT INBRED STRAINS OF MICE AND THEIR HYBRIDS

	DBA_f	$C_{57}BL$	O_{20}	C_3H_f	$F_1(C_{57}BL \times DBA_f)$	$F_1(O_{20} \times DBA_f)$
Average Lifespan	598	621	684	685	711	786
Mammary carcinoma	4%	0%	0%	6%	2%	3%
Leucosis	3%	5%	0%	3%	7%	9%
Long papilloma	0%	0%	10%	1%	2%	5%
Uterus sarcoma	3%	2%	0%	1%	6%	4%
Ulcers of the skin	3%	0%	0%	0%	0%	0%
Hepatoma	0%	0%	0%	4%	0%	0%
Ovarian tumours	0%	0%	0%	1%	0%	2%
"Senile decrepitude"	87%	93%	90%	84%	83%	76%

has first been described by Dunn (1944). This senile amyloidosis is dependent upon dietary and genetical factors, which have been analysed by Heston and co-workers (Heston and Deringer, 1948; Heston, Larsen and Deringer, 1945). The genetical aspects may be illustrated as follows:

The DBA_f strain has a high incidence of amyloid in the adrenals and in the ovaries (about 90 per cent in mice over the age of 18 months), while the kidneys are usually only slightly affected or not at all.

The O_{20} strain is practically free of this amyloid degeneration. In the adrenals it was seldom seen, while the kidney only

exceptionally showed amyloid disease. The $O_{20} \times DBA_f$ hybrids, however, are very frequently affected. Above an age of 20 months various organs are increasingly affected, the incidence in the adrenals and in the kidneys being around 90 per cent at 24 months.

The amyloid degeneration of the mouse kidney may be briefly described as follows: while amyloid may occur in the glomeruli, its most frequent site is the interstitial tissue of the renal papilla. The amyloid deposits here lead to obstruction of the Bellini ducts. Infection may secondarily occur, and the final result is atrophy and degeneration of the renal tubules, often in wedgelike areas. For this process Dunn has proposed the name "papillo-nephritis". This degeneration may be accompanied by cystic dilatation of the tubules and of the Bowman capsules. According to the occurrence and degree of these dilatations, two varieties of the affection are discernible macroscopically: the cystic and the atrophic form.

The occurrence of these dilatations also depends upon genetical factors. The cystic form of papillo-nephritis is found in $C_{57}BL$ mice and is most frequently found in $C_{57}BL \times DBA_f$ hybrids. In $O_{20} \times DBA_f$ hybrids, on the other hand, the atrophic form is usually found.

These lesions of the kidneys are but one instance of senile changes leading to death. Many others certainly remain to be analysed. In these investigations a comparative analysis of the functions of young and old organs and tissues is necessary.

Transplantation Experiments

One of the outstanding advantages of inbred strains is the possibility of transplantation of tissues and organs from one animal to another, either within the inbred strains or from an animal of an inbred strain to one of its F_1 hybrids. The reverse, i.e. transplantation of organs and tissues from an F_1 hybrid into one of its parent strains is not possible.

The use of this transplantation method can be demonstrated with the transplanted ovaries, in which the function of the

graft can be followed from day to day with the aid of vaginal smears. The various aspects of the transplantation of the ovary have been exhaustively discussed by Krohn (1955). In our own experiments with subcutaneous transplantation of ovaries into ovariectomized females, a completely normal oestrus cycle was found which lasted in most cases nearly as long as in the animal with the ovaries *in situ* (Boot, 1956). Complete normal hormonal function could also be proved by successful transfer of fertilized ova into the animal with the subcutaneous transplant.

The technique of organ transplantation can also be used for the study of differences in ageing processes of organs in different inbred strains. For instance, when we transplant ovaries of two inbred strains to their F_1 hybrid, and thus to the same environment, we can determine which ageing processes are inherent in the ovary itself and which are dependent on changes in other organs, e.g. in the hypophysis.

There is one point which has, however, to be taken into consideration, when we transplant tissues or organs from an animal of an inbred strain to an F_1 hybrid: i.e. the possibility that the tissues may change in the new host by adaptation or mutation. This change has been found by Barrett, Deringer and Hansen (1953) with transplants of malignant tumours from inbred strains to F_1 hybrids.

Another question which can be investigated with transplantation experiments is that of the lifespan of the different organs. Experiments with old ovaries transplanted into young animals showed that a prolongation of the hormonal function is not possible far beyond the ordinary lifespan of the ovary.

Similar approaches are possible for other organs, although it must be borne in mind that not all transplantations are as successful as those with ovaries.

Transplantations of the testes are only successful when the transplanted testes are taken from 3-day-old animals. More adult testes, even when stripped from their connective tissue capsule, show after transplantation no functional activity.

Parabiosis

Another method which apparently has not as yet been much used in gerontological research is that of uniting two animals in parabiosis. Inbred strains are indispensable for this method. If we unite in parabiosis an old animal with a young one, it is possible, e.g., to study the gonadotrophic function of the hypophysis of the old animal, or *vice versa* the influence of the young hypophysis on the old gonads. The after-care of these parabiotic animals is most essential. In our laboratory the method of putting an older female which has had some litters into the cage of the parabiotic animals as a nurse has appeared to be extremely useful.

Conclusion

A survey has been given of the use of inbred strains in experimental gerontology research. It is beyond doubt that inbred strains and their F_1 hybrids are indispensable for the investigation of many gerontological problems.

REFERENCES

Barrett, M. K., Deringer, M. K., and Hansen, W. H. (1953). *J. nat. Cancer Inst.*, **14**, 381.

Billingham, R. E., and Parkes, A. S. (1955). *Proc. roy. Soc.* B., **143**, 550.

Boot, L. M. (1956). *Acta physiol. pharm. néerl.*, **4**, 565.

Boot, L. M., and Mühlbock, O. (1954). *Acta physiol. pharm. néerl.*, **3**, 299.

Comfort, A. (1956). The Biology of Senescence. London: Routledge and Kegan Paul.

Dunn, Th. B. (1944). *J. nat. Cancer Inst.*, **5**, 17.

Grüneberg, H. (1954). *Nature, Lond.*, **173**, 674.

Heston, W. E. (1945). A Symposium on Mammary Tumors in Mice. Publ. Amer. Ass. Advanc. Sci., No. 22, p. 55.

Heston, W. E., and Deringer, M. K. (1948). *Arch. Path.*, **46**, 49.

Heston, W. E., Larsen, C. D., and Deringer, M. K. (1945). *J. nat. Cancer Inst.*, **6**, 41.

Krohn, P. L. (1955). Ciba Foundation Colloquia on Ageing, I, 141. London: J. & A. Churchill Ltd.

LEE, S. VAN DER, and BOOT, L. M. (1955). *Acta physiol. pharm. néerl.*, **4**, 430.
MÜHLBOCK, O. (1950). *Ned. Tijdschr. Geneesk.*, **94**, 3747.
MÜHLBOCK, O. (1951). *Acta Un. int. Cancr.*, **7**, 351.
MÜHLBOCK, O., TENGBERGEN, W. VAN EBBENHORST, and RIJSSEL, TH. G. VAN (1952). *J. nat. Cancer Inst.*, **13**, 501.
RUSSELL, W. L. (1941). Biology of the Laboratory Mouse, p. 325. Philadelphia: Blakiston Co.
THUNG, P. J. (1955). Old Age in The Modern World, p. 150. Edinburgh: Livingstone.
THUNG, P. J. (1956). Symposium on experimental Research on Ageing, Basel: Birkhäuser-Verlag.
THUNG, P. J., BOOT, L. M., and MÜHLBOCK, O. (1956). *Acta Endocr., Copenhagen*, **23**, 8.
THUNG, P. J., and RIJSSEL, TH. G. VAN (1954). *Ned. Tijdschr. Geneesk.*, **98**, 2028.

DISCUSSION

Olbrich: As you have shown, kidney infections very frequently occur in mice. We don't know how long infection has been present before amyloidosis appears. You work a fairly long time with a nephritic animal. The pyelonephritis which you have shown is often seen in humans. Rats or mice frequently get monilia—and other infections which last a long time and lead to the development of amyloidosis. Do you know how long the infection has been present? It is peculiar that the majority of your animals suffer from "senile decrepitude"; I think these animals are suffering from amyloidosis which is the result of a long-standing infection of the kidneys. In other words you are experimenting with uraemic and not normal animals.

Mühlbock: We do not consider that the amyloidosis is a consequence of an infection. We think it is a primary amyloidosis, and that it is related to a special genetic constitution. Why should we have such infections in one strain of mice and in another strain no infection at all? Moreover, in the kidneys amyloid infiltration comes first, and inflammation is secondary.

Olbrich: The only point is this: primary amyloidosis never appears in the kidney in humans.

Mühlbock: It may, exceptionally. The frequent renal localization in mice is genetically conditioned. There are more of these peculiarities in comparative gerontology: I have never seen arteriosclerosis in the mouse and yet it is seen in the human being. We feel that senile amyloidosis is the cause of death in these animals.

Krohn: Amyloidosis is very unlikely to have occurred in the ovary as a result of infection.

Olbrich: You don't get it in ovaries.

Krohn: Yes, you do in mice.

Mühlbock: Yes, practically in all organs, but the distribution over various organs is different in different strains.

Gillman: Is there any sex difference in amyloidosis?

Mühlbock: We found no such difference, except in organs which themselves show sex differences as, for instance, the adrenal cortex. However, because of our work on mammary cancer we do most work with female mice and we have much more experience with female animals than with males.

Gillman: I asked that question because my brother, Prof. Joseph Gillman, and Dr. Christine Gilbert have recently described quite a high incidence of spontaneous amyloidosis in the female baboon. This they consider in some way to be related to the cyclical absorption of the sex skin material (Gillman, J., and Gilbert, C. (1955). *Acta med. scand.*, **152**, suppl. 306, 155). One wonders whether the cyclical alterations in the connective tissues associated with ovarian activity may not contribute towards the amyloidosis in Dr. Mühlbock's animals.

Parkes: I was fascinated by many of the experiments which Dr. Mühlbock described, and I would like to congratulate him.

The young mice which received the old ovaries as a graft gradually went off cycle; what was the histological condition of the graft by that time?

Mühlbock: We don't find much of them; there is some cell growth remaining.

Parkes: What was the histological condition of the old ovaries when they went in as a graft? Was the failure of the ovary to keep up its endocrinological secretion after grafting due to exhaustion of the supply of eggs or to something else?

Mühlbock: I think it was due to exhaustion.

Parkes: The grafting would have destroyed a lot of the remaining eggs and a few cycles might exhaust the ovary altogether.

Mühlbock: I think that is exactly what happens.

Franklin: Dr. Mühlbock, what was the safe distance apart for your animals not to get pseudopregnancy as a result of smelling one another?

Mühlbock: If you just take a cage that has been soiled by the animals and put an animal in there it will become pseudopregnant.

Best: That will involve an estimation of the acuity of the sense of smell?

Franklin: Yes, it does.

Krohn: I am not at all certain that you are right when you suggest that the ovarian function stops when there are no more ova. It is my impression that the ovaries of old animals which have gone on cycling for some considerable time after they have become infertile still look quite healthy histologically. There is plenty of granulosa-like tissue but there are no ova or oöcytes in the ovaries themselves. I think the two functions do not stop at the same time; they can be separated in time and probably by a period of six or seven months or so.

Mühlbock: However, we found that oestrus cycles may continue while fertility has stopped. This cessation of fertility appears to be due to ovulatory failure, and in the ovaries no normal follicles are found.

Krohn: Yes, but I believe those animals have not or may not have any ova at all in the ovary. It is not that they are ovulating inadequate ova, but that if you look at the ovaries of old animals which are still

cycling apparently normally, you can find absolutely no form of oöcyte throughout the whole serial sections of the entire animal's ovaries.

Parkes: Dr. Krohn, you are suggesting that the ovary will continue to produce oestrogen, or cyclically to produce some semblance of an oestrus cycle for many months after the last egg has disappeared.

Krohn: I am indeed, exactly.

Parkes: I have been reading a number of papers which imply that the disappearance of eggs from the ovary, as by radiation, necessarily means that the cycles would stop fairly soon.

Krohn: The situation is quite different when you irradiate the ovary. I am referring here to work by Mandl and Zuckerman (1956, *J. Endocrin.*, **13**, 243). Following irradiation of the ovary you can destroy all the oöcytes very rapidly. Such animals go on having irregular oestrus cycles for up to 6 weeks and then go into constant vaginal oestrus for as long as 100 days. What you are doing in irradiation is damaging every component of the ovary and you are destroying all those tissues which could go on secreting oestrogen for very long. Once the lifespan of anything that you have left behind after irradiation is over, in 60 days or so, then there is nothing left from the ovary to go on producing oestrogen; and I imagine that in a very old mouse you would get the same situation: first of all there are no ova left, then there is no luteal tissue or granulosa cell tissue, or whatever it is, left and finally there is nothing left and you would find permanent dioestrus or anoestrus.

Parkes: What is your proof that, after irradiation with a moderate dosage, anything would have been destroyed except the eggs?

Krohn: I have no proof of that. That is what I would presume has happened. I cannot believe that the dose of radiation can be so finely adjusted, and certainly no attempt has been made to adjust it finely, so that it would just kill the oöcytes and not kill any of the other tissue in the ovary.

Parkes: By using a strict dosage you can avoid damaging almost any other cellular bodies.

Krohn: Dr. Mühlbock, when you are doing your breeding to get an inbred strain I think it is important that your choice of breeding pairs in each generation should be at random. In selecting pairs from one generation to another one often says "That is a good-looking mouse, and that one is, and we shall take those as our breeding pair"; one is then, in effect, selecting residual heterozygosity and it will take far longer to get homozygosity than just taking a pair entirely at random. I believe that the fact that the strain was not inbred sufficiently has proved to be an important factor in some work on skin grafting.

Mühlbock: Yes, I agree, but pregnancy is another factor in selectivity. All our strains are inbred for one hundred generations or more and so we are relatively sure that we have really pure strains. I would like to stress a point that most people do not realize: there must be no relaxation of inbreeding, that is an essential point.

Best: You use parabiosis as a test for purity of strain? With what do you correlate the ability of the animal to live in the parabiotic state? What do you look at in the animals?

Mühlbock: We just see that they are healthy and live as long as ordinary mice.

Best: Have you made the parabiotic union at different phases of inbreeding?

Mühlbock: Yes.

Best: And does it always go well after twenty inbreedings?

Mühlbock: Yes, but it is an absolute requirement that they must be absolutely genetically identical.

Verzár: Have you united parabiotically old and young rats, and how was the survival then? For instance, if you unite a 6-month- and a 24-month-old one, what is the result?

Mühlbock: They live quite happily together for 3–4 months and die at 28–30 months.

Best: If you did not have this parabiotic test, how would you know if you had a pure strain?

Mühlbock: By the transplantability of one organ to another.

Tunbridge: When you mate a 6-month- to a 24-month-old animal and they die at 28 or 30 months, if there is no cancer what is the spread of amyloid disease in parabiosis? Is it roughly the same percentage in all organs, young and old, or is it just in the ovary?

Mühlbock: We have not looked at that in parabiotic animals. In old single animals the distribution of amyloid shows strain differences.

Friedman: Dr. Mühlbock, with regard to the union of a young animal with an old animal (taking 24 months as the age of the old one), do you have any information about what would happen if you considered the onset of age to occur sooner, for instance at the end of fertility? At 24 months you may be trying to reverse something which may be well along and irreversible, whereas actually loss of fertility, if that were taken as the index, would occur a good deal earlier. I wonder if the same idea might not be extended to this question of the transplantation of the ovary. In other words, is there a point of irreversibility if one waits too long before attempting to study the effects of the younger tissues on the older ones?

Mühlbock: We controlled, of course, the function of the ovaries in the 24-month-old animal before transplantation. We took the ovaries only if there was still an oestrus cycle, which means that they were still functioning. We were, of course, interested in the eternal question: "What grows old first, hypophysis or ovary?" We correlated ovarian function with the oestrus cycle. There is very little margin to do this type of experiment. If the function of the ovaries stops in an animal, say, at the age of 26 months, then there is the possibility that the animal will live for two or three months longer and you can do this type of experiment and can see if the hypophysis is still producing gonadotrophic hormone or not. So far, we can say that the hypophysis produces gonadotrophic hormone.

Friedman: While it is quite true, I think, that the hypophysis is still functioning, our experiments suggest that there is a fair amount of fall-off in neurohypophyseal function with age; this fall-off, while it is very advanced at 24 months, can be detected a good deal earlier than

that. We have just started some experiments to see what would happen with replacement therapy, but I think we ourselves have been somewhat misled by waiting too long. If we take a 24-month-old rat, we are surely dealing at that point with an animal in which replacement cannot be too successful since the neurohypophyseal failure seems to start a good deal earlier, possibly at 16 or 18 months. I am really just wondering about the course of the functional decrements in age, i.e. instead of thinking of a sharp fall-off into an old age period, perhaps one should look for an earlier point of inflection in the curve of ageing, which might really be the critical time.

Mühlbock: What we do believe from our experiments is that there is no point where endocrine activity approaches zero values. What really occurs, in fact, is some imbalance in the hormonal system which develops very gradually. I think that in each case you have to study this imbalance. We have studied the hypophysis, and the dominant function of the anterior hypophysis in old mice was prolactin production.

Landowne: Do you find that the ability of animals who will accept parabiosis or transplantation changes with age in your material? Are there a certain number of successes and failures, depending for instance on whether you transfer a young tissue to an older animal or *vice versa*?

Mühlbock: If you use a "take" as a criterion, then there is no difference, but of course there is some relation to time. So, I always say that if you do a transplantation you must have 100 per cent success.

Landowne: This applies to the old animal as well?

Mühlbock: Yes, but if you take the development of an ovarian tumour, this will develop in three weeks in a young animal, and maybe you will have to wait six weeks or so before it develops in an older animal.

Danielli: I am not really convinced that, beyond all question, it is the sense of smell which is involved in these very interesting experiments, at least not from the evidence you have presented. It seems to me that it is possible that some other stimulus is involved but which is only operative if the olfactory bulbs are present. And whilst I agree that it is rather improbable that this is so, nevertheless the changes you observed were so large that it would be nice to have absolute proof. Have you any evidence that simply "piping the smell", as it were, of a lot of animals, is sufficient?

Mühlbock: Yes, we have kept a number of animals in one cage, and when after a week we took them out, took them to another room and put an animal in the cage, the result was just the same.

Danielli: But then you transferred rather more than simply an olfactory stimulus.

Mühlbock: Yes, I completely agree.

Bourlière: A few years ago Le Magnen (1951, *C. R. Soc. Biol., Paris*, **165,** 851) carried out some experiments on the olfactory abilities in white rats, which fit in very well with Dr. Mühlbock's experiments. He found not only that rats are able to recognize both sexes on olfactory grounds but that they can also discriminate between a female in heat and one that is not.

Danielli: Yes, it is not that I am questioning the likelihood that Dr. Mühlbock's is the right explanation. But it comes so close to being proof that one would like to see the proof actually established.

Parkes: Have you any idea of how smelling other individuals makes them pseudopregnant?

Mühlbock: We think that it is a mechanism which is set in motion once a certain level of stimulation is reached. Some centre in the nervous system, which may be stimulated along several pathways, then releases some factor which in its turn causes luteinizing hormone or something like that to be released by the hypophysis.

Danielli: There is a somewhat analogous situation in locusts connected with the change from the solitary to the swarming phase. I am not sure whether the mechanism has been really established there. I believe that there is a certain amount of evidence that there must actually be contact between the locusts; it is not sufficient that they see one another or smell one another.

Another point I should like to discuss is whether you really have worked with homozygous strains. I should have thought that that was highly improbable. It would mean that there were no hidden recessives in your stock and that strikes me as being improbable. It does seem more likely that you managed to get a certain number of strains in which certain groups or sets of the genes turn up, are really viable, and other sets of the genes, so to speak, die off in the early stages, resulting in death in early embryonic stages or even before fertilization. Now, a situation like that might lead to a strain in which most, if not all, of the animals would be very closely similar in their genetic constitution, but there is a big jump from that situation to one where a pair of animals is genetically identical.

Mühlbock: Would you agree with 99·999 per cent?

Danielli: I would not agree with any numerical assessment unless you had appropriate evidence.

Bourne: You said that in your parabiotic experiments with the young and the old, the old animal dies within a few months; does the young animal die with him?

Mühlbock: Yes, it does; but if you separate them the young one will survive.

TWIN DATA ON THE GENETICS OF AGEING*

Franz J. Kallmann

Department of Medical Genetics, New York State Psychiatric Institute, Columbia University

Despite the intricate and continuous interdependence of genetic and non-genetic components of personality formation, the twin study method makes it possible to appraise the effect of heredity on measurable behaviour variations at all stages of the human lifespan, inclusive of the senium. The technical efficacy of the method is matched by its economy and versatility as a sampling procedure, and extends to population studies which call for a comparative analysis of individual health and survival values. These procedural advantages are most apparent in the investigation of traits requiring intra-family and longitudinal comparisons under controlled conditions, and personal contact with families from various population groups, including some whose private affairs might not otherwise be open to study.

The popular notion that the adjustive and ageing patterns of one-egg twins resemble each other chiefly because of unusual similarity in their early environments has yet to be substantiated. If confirmed, the argument would only strengthen rather than weaken any correctly formulated genetic theory. Psychodynamic concepts, too, are predicated on the premise that man is selective in regard to important aspects of his life experiences (Alexander, 1956) and takes pride in being responsible for his own formula of adjustment, before and during the period of senescence.

From a biological standpoint, the process of ageing has been

* Presented at the Third Ciba Foundation Colloquium on Ageing, as the eighth consecutive report on the progress of a study which has been supported by grants from the Rockefeller Foundation (1945–1951) and the Division of Research Grants of the National Institutes of Health (1952–1956).

defined as a gradual loss of ability to maintain a constant level of physiological equilibrium. Hence, it is the ability to withstand the ensuing impairment of health that determines survival to an advanced age. This capacity is known to be so variable that the many observed differences from one person to another strongly indicate the operation of gene-controlled phenomena (Kallmann, 1956 *a* and *b*; Kallmann, Aschner and Falek, 1956). That the potentiality for a long life, derived from a fortunate combination of health-conferring genes, can be modified by adverse life conditions in no way lessens the significance of this fundamental biological hypothesis.

Genetically, a distinction is made between the effects of gene-specific processes causing premature or other pathological disturbances in the last sector of the human life cycle, and those of general genetic phenomena which produce differences in basically positive health and survival values (Kallmann, 1953). The symptoms observed in the first group of disorders are likely to be the result of one major mutant gene that follows the single-factor type of inheritance.

Seen in the second group of variable patterns of adjustment are gradations in normal ageing potentials which result from the interaction of several or many genes and are therefore ascribable to the multifactor or polygenic mode of inheritance. Extreme variations determined in this manner (through the accumulation of short-life genes) are apt to produce deviations from the mean health status of an ageing population, which are classifiable as pathological. Some of these deviations may be so clearly pathological as to be indistinguishable from either a single-factor type of disturbance or a so-called phenocopy (a non-hereditary variation simulating the phenotype of a mutant gene).

Still another group of minus variations in adjustment to ageing is due to gene-controlled deficiency states, physical or mental, which arise before the senescent period but tend incidentally to alter the adaptive plasticity of ageing persons. In this category are the major psychoses, specific metabolic or endocrinopathic disorders, and various types of intellectual

subnormalcy and emotional instability, including schizoid personality traits, compulsive drinking patterns and the like.

Exact genetic information about the manner in which presenescent maladjustment affects adaptability to ageing has yet to be obtained. Longitudinal studies are needed to demonstrate how specific traits tend to complicate, or are complicated by, the ordinary phenomena of old age such as those described by McFarland (1956) and various Russian investigators (Bogomolets, 1938; Gakkel and Zinina, 1953). Since the few studies made in this field have been related largely to chronological rather than biological age, present appraisal of changes due to ageing can be no more than a gross approximation.

It would be difficult, for instance, to determine whether complications in the later stages of chronic pathological conditions, such as hypertension or hypothyroidism, arise in the period of senescence by coincidence or as the result of a causal constitutional relationship. In the case of a gene-specific underproduction of the thyroid, the connecting link may be a disturbance in cholesterol metabolism which affects both physical and emotional equilibrium.

On the whole, the impact of the senium may be expected to intensify pre-existing maladjustment. Old schizophrenics and mental defectives tend to deteriorate, and alcoholics almost always show a marked decline in tolerance and general resistance.

Regarding emotional maladjustment which phenomenologically falls into the involutional period, the relationship between the effect of advancing biological age and declining adaptability is demonstrated by persons distinguished by a schizoid type of behaviour pattern. There is substantial evidence in support of the theory that the schizoid personality structure is that of a heterozygous carrier of the schizophrenic genotype with an inadequate degree of general constitutional resistance (Kallmann, 1952). The presenescent traits most commonly associated with this type are rigidity, compulsiveness and oversensitivity. Along with the cumulative emotional

strain arising from increasingly conspicuous signs of ageing, these traits tend to lead to painful experiences in interpersonal relationships, gradually overtaxing the adaptive defense mechanisms.

When a psychotic break with reality occurs, its symptoms are characterized by the inability to find constructive avenues for releasing anxiety generated by involutional changes. Aetiologically, however, it is obvious that the causes of such an involutional psychosis are multiple and always include a long history of emotional instability determined genetically. Admittedly, the biochemical correlates of this deficiency in adjustive plasticity are still very much in need of clarification.

Similar uncertainties exist with respect to the genetic aspects of those pathological conditions which are specific to the period of senescence. Sufficient information is not even available regarding the least common and most easily recognizable disorders known as Pick's, Alzheimer's and Jacob-Creutzfeldt's diseases. Here, gross and relatively circumscribed brain lesions develop so dramatically and prematurely that from a genetic standpoint it would be possible to think in terms of specific disturbances produced by the effect of single mutant genes (Essen-Möller, 1946; Jacob, Pyrkosch and Strube, 1950; Klöpfer, 1956; Malamud and Waggoner, 1943).

Theories of simple dominance or recessiveness have been advanced not only for these three special types of presenile brain atrophy, but also for essential hypertension and cerebral arteriosclerosis and the total group of disorders called senile dementia. However, histologically verified family data are still so scarce in this area that it would seem inadvisable to eliminate polygenic modes of inheritance, even in the fairly well-studied groups of Alzheimer's and Pick's diseases. According to Sjögren, Sjögren and Lindgren (1948), the theory of a simple dominant mode of inheritance has been more clearly substantiated in Pick's than in Alzheimer's disease. The observed morbidity rates for the parents and sibs of affected persons are 19 and 6·8 per cent in Pick's disease, and 10 and 3·8 per cent in Alzheimer's disease.

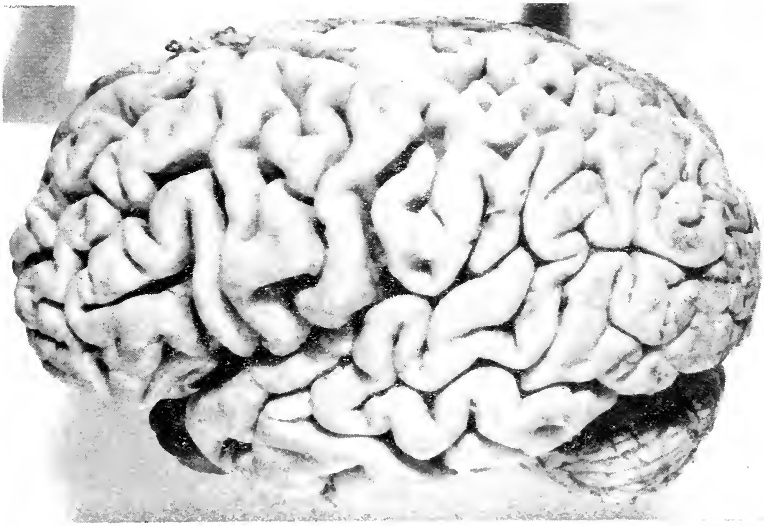


FIG. 1. Generalized presenile atrophy of the brain (Alzheimer).

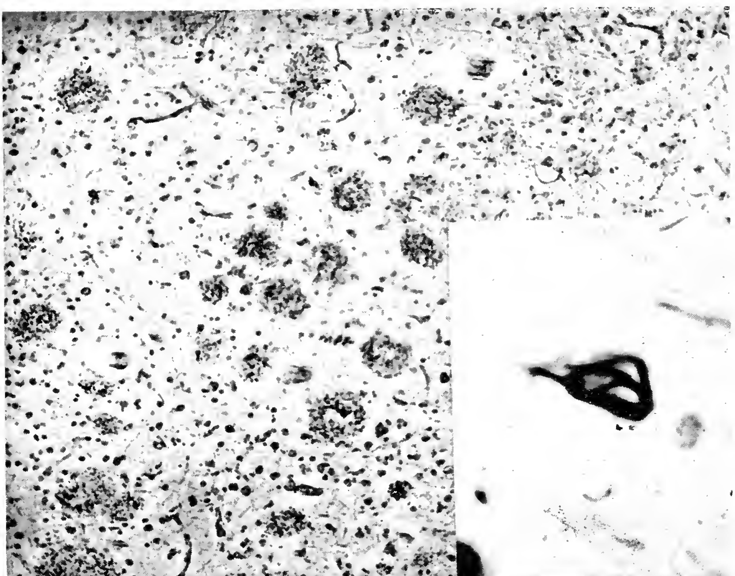


FIG. 2. Senile plaques and neurofibrillary degeneration in Alzheimer's disease.

[*facing page* 134

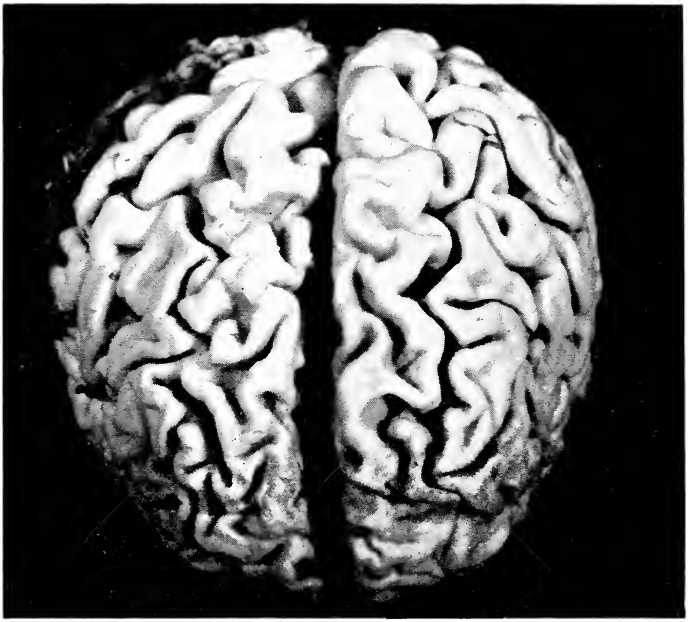


Fig. 3. Circumscribed presenile atrophy of the brain (Pick).

Fig. 4. Cytoarchitectural disorganization in Pick's disease (Nissl Stain).

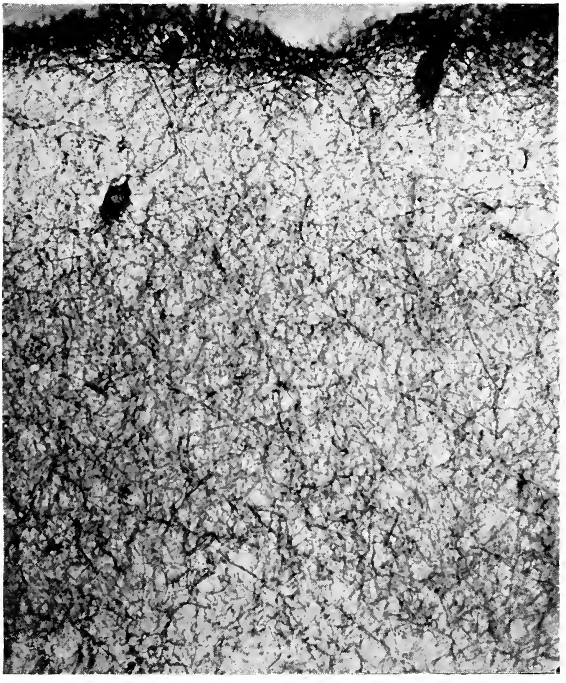


Fig. 5. Cortical gliosis in Pick's disease (Holzer method).

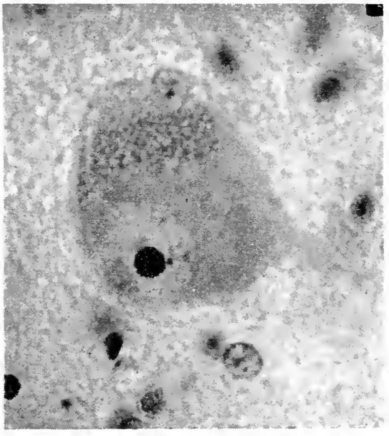


FIG. 6. Accumulation of lipid material in ballooned cell (Pick).

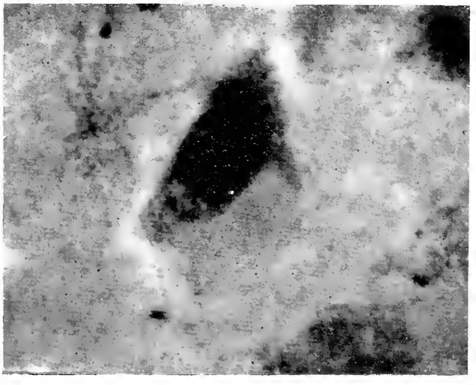


FIG. 7. Pick's cell type with eccentric nucleus.

Virtually nothing is known about the gene-specific metabolic disturbances that may be at the root of these two types of early brain atrophy. Conjecturally it may be stated that the given disturbances are likely to differ as much in character as do the structural changes produced by them. It is regrettable that our search for verified twin cases of either type has not been successful, but it may be useful here to describe some of the histopathological differences observed between the two conditions.

In Alzheimer's disease (Fig. 1), the atrophy of the brain, although most pronounced in the temporal and frontal lobes, is rather generalized. On the whole, the narrowing of the gyri and the widening of the sulci are more severe than the common atrophic changes associated with the senium. Microscopically, there are early and marked degenerative changes, and the cytoarchitecture is so disorganized that some areas are entirely lacking in nerve cells.

Application of the silver impregnation method discloses an abundance of senile plaques, and typical neurofibrillary degeneration is seen in the greatly magnified cell inserted (Fig. 2). Parenthetically it may be noted that these degenerative changes are not argentophilic.

Compared with the atrophic changes observed in Alzheimer's disease, the atrophy of the brain in Pick's disease (Fig. 3) is more circumscribed and usually most severe in the frontal lobes. The postcentral and parietal regions tend to be better preserved. Microscopically, the cytoarchitecture of the frontal lobe may show complete disorganization (Fig. 4). In this instance, there are scarcely any nerve cells left, and certainly fewer than in Alzheimer's disease.

Using Holzer's method for glia fibres (Fig. 5), one finds a marked cortical gliosis, a symptom which distinguishes Pick's from Alzheimer's disease. Greatly magnified, a typical cell described by Pick (Fig. 6) is characterized by an eccentric nucleus and a ballooned appearance of the cellular body.

Within the cell body (Fig. 7), one sees a massive accumulation of lipid material which is highly argentophil in character.

This finding and the absence of both senile plaques and neurofibrillary changes are usually sufficient to differentiate Pick's from Alzheimer's disease. In fact, the degenerative processes in Pick's disease are so peculiar that, genetically, they would seem to point to a very specific metabolic disorder which has yet to be identified.

For this purpose, broad interdisciplinary family investigations of the kind now in progress at Tulane University (Buchwald, 1956; Klöpfer, 1956) will be required. From a genetic standpoint, linkage and pleiotropism studies may be helpful in an attempt to locate the chromosomal position of the defective gene or genes and to detect the presence of such gene effects in persons displaying no overt manifestation of the disease process. In biochemical research, chromatographic and electrophoretic methods may provide essential information about the part played by amino acids and serum lipoproteins.

Much additional genetic work is also needed in those disorders in later life in which cerebrovascular changes are the predominant feature. Obviously, arteriosclerotic symptoms are developed much earlier and more severely by some persons than others, and equally variable is the ability to withstand cerebral damage (Adlersberg, Parets and Boas, 1949). Clinically, it has also been established that atherosclerosis is a disease in itself and not an ordinary concomitant of ageing. In fact, it has been suggested by some investigators that atherosclerosis may actually be two distinct diseases: the first due to defects in cholesterol metabolism or circulatory functions; the second resulting from a breakdown of the structure of the elastic elements in the media of arteries, accompanied by calcification. The increase in deposited calcium has been shown to be associated with an increase in some amino acids, which in turn may be related to the action of elastase, a pancreatic hormone (Glass, 1955).

In any case, it is particularly in the presence of a specific disturbance in lipid metabolism (known as primary hypercholesterolaemia) that a familial trend is found toward such

pathological conditions as coronary artery disease, essential hypertension and cerebral arteriosclerosis (Friedman, Rosenman and Byers, 1955; Wilkinson, 1950). Some investigators regard dominant genotypes as the cause not only of a specific predisposition to cerebral arteriosclerosis, but also of its particular localization and frequent combination with nephrosclerosis (Søbye, 1948). However, despite the present evidence for a gene-specific metabolic error being the basic cause of hypertensive disease, many of the aetiological aspects of this serious affliction of the ageing are still unsolved.

Equally doubtful is the theory that the total group of senile dementias may be due to the effect of a dominant genotype of low penetrance (Cresseri, 1948; Meggendorfer, 1939). Either one or two dominant genes have been assumed to be involved, one controlling longevity and one producing the pathological changes associated with senile dementia. According to the results of our study of ageing twin family units, senile psychoses seem more adequately explained by an age-specific intensification of long-existing but minor deficiencies in general emotional adjustment than by a single genetic factor causing a specific type of psychopathology. This theory implies that the genetic components in the aetiology of a senile psychosis consist of polygenically determined variations in age-susceptible personality traits, a generally reduced level of adaptive plasticity, and those gene-specific biochemical phenomena controlling growth and decline.

Graded differences in general ageing and longevity potentials have been demonstrated by family statistics (Dublin, Lotka and Spiegelman, 1953; Gianferrari, 1954; Jalavisto, 1951; Pearl and Pearl, 1934) as well as by twin studies (Kallmann, Feingold and Bondy, 1951; Kallmann and Sander, 1948; von Verschuer, 1954) and are most certainly polygenic in origin. Longitudinal twin data show that compared to the limited degrees of similarity between two-egg twins or ordinary sibs, all measurable similarities between ageing one-egg twins are consistently more pronounced, frequently in spite of very different environments. The observed similarities

extend to physical and mental signs of ageing, social adjustment, intellectual performance, and its rate of decline. The comparative histories of two typical twin pairs may help to illustrate this point.

The twin brothers in Fig. 8 belong in the series of two-egg pairs with very similar life conditions. Reared on a farm, they attended a rural school together, and then became prosperous farmers in the same district. When they were in their early thirties, each married a local girl and had one daughter. However, despite the similarity of their lives, they developed rather disparate personalities and patterns of ageing.

The younger-looking twin on the left, described as prankish, excitable and somewhat extravagant, complained of ill-health for thirty years before retiring at age 70. The other twin, who still runs his farm on his own, has always been placid, frugal and self-sufficient. He takes pride in never having seen a doctor, except for an appendectomy.

As to general intelligence, this twin has maintained a two-point advantage over his brother, with vocabulary scores of 23 and 22 at the ages of 71 and 78. But in this period of seven years, he has deteriorated from a score of 63 to 30 on the tapping test, and from 25 to 17 on the digit symbol test. The corresponding decline in the twin with the slower ageing patterns has been only from 78 to 67, and from 27 to 21, respectively.

The one-egg twins in Fig. 9, on the other hand, are still remarkably alike, both physically and mentally, although their fortunes have differed considerably. Both were employed in factories, married at a young age, and fathered six children each. At 78, one is maintaining a stable home life in a suburban community where he is still active as a cemetery worker. The other neither works any longer, nor does he have a home of his own. For forty years he has been separated from his wife, for whose sake he had changed his religion.

Nevertheless, both twins are in good physical health and blissfully unaware of their equally advanced state of senile

Fig. 8. Dizygotic twins at the ages of 23 and 78.

[*facing page* 138

Fig. 9. Monozygotic twins at the ages of 25, 70, and 78.

deterioration. In the last seven years, their digit symbol scores have declined at a similar rate, from 13 to 2 and from 11 to 1. Interestingly enough, even the mistakes they make in substituting symbols for digits are precisely the same.

These two sets of aged twins are among the 72 paired survivors of a series of 120 same-sexed pairs, tested psychometrically in 1947 and, where possible, retested with the

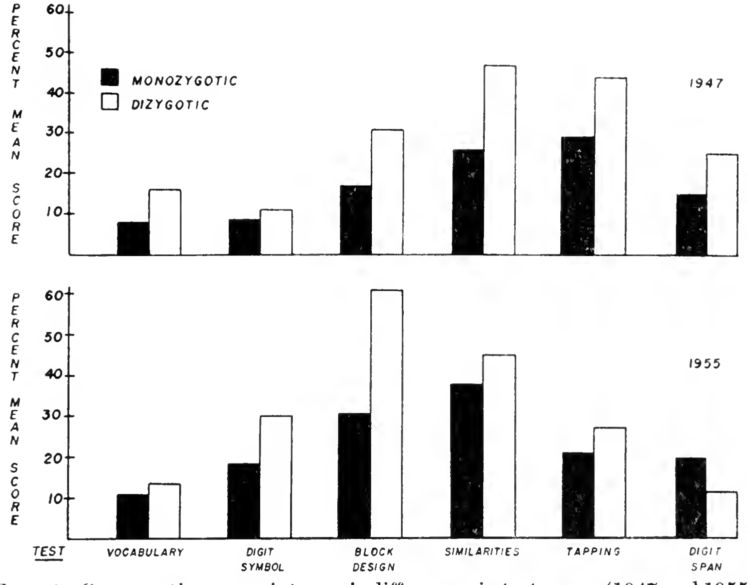


FIG. 10. Comparative mean intra-pair differences in test scores (1947 and 1955).

same battery in 1955. It is indicated by the results of this longitudinal study, summarized diagrammatically in Fig. 10, that the mean intra-pair differences in the test scores of two-egg twins consistently exceed those of one-egg twin partners. The detailed test data will be presented at the International Congress of Human Genetics in Copenhagen, as long-needed evidence for the essential part played by genetic factors in intellectual performance until the period of senescent decline (Feingold-Jarvik, Kallmann, Falek and Klaber).

In line with clinical and psychological observations, it is shown by comparative data on the length of life of senescent twins (Table I) that basic variations in the human lifespan depend on gene-specific health and longevity potentials. In those pairs of our sample where both twins died after age 60,

Table I

BIENNIAL MEAN INTRA-PAIR LIFESPAN DIFFERENCES IN SAME-SEXED TWIN PAIRS OVER 60

	YEAR OF ANALYSIS	NUMBER OF SAME-SEXED PAIRS		INTRA-PAIR LIFE SPAN DIFFERENCES EXPRESSED IN MONTHS		
		ALL PAIRS	BOTH DECEASED	MALE	FEMALE	TOTAL
MONOZYGOTIC	1948	237	32	47.6	29.4	36.9
	1950	415	68	42.9	31.2	36.7
	1952	513	76	40.7*	30.7*	35.7*
	1954	513	78	40 7*	31.6*	36.0*
DIZYGOTIC	1948	696	36	89.1	61.3	78.3
	1950	1071	70	79.1	63 2	71 8
	1952	1226	86	79.1*	69 5*	73 7*
	1954	1226	102	69.5*	79.1*	74.6***

* Significant at 1% level

** All opposite-sexed pairs over 60: 106.0 months

the mean intra-pair lifespan difference continues to be much smaller in one-egg than in two-egg pairs. The present total mean difference varies from 36·0 months in the one-egg group to 74·6 months in the two-egg group of the same sex, and to 106·0 months in dizygotic pairs of opposite sex. In same-sexed pairs, this difference is about the same for both sexes, despite the shorter lifespan of the male. It may also be mentioned that one-egg twin partners are more than twice as

similar in causes of death as two-egg pairs of the same or opposite sex.

The gene-specific basis of longevity and general health potentials in old age is confirmed by a comparison of the lifespans of the sibs of senescent twin index cases with those of their parents (Table II). The most striking result of this analysis is the evidence for a direct relationship between parental age and the lifespan of the offspring. The effect

Table II

EFFECT OF PARENTAL AGE ON MEAN LIFESPANS OF SENESCENT TWINS AND THEIR SIBLINGS

	MOTHER DIED			FATHER DIED			BOTH PARENTS DIED		
	BELOW 55	55–69	70 AND OVER	BELOW 55	55–69	70 AND OVER	BELOW 55	55–69	70 AND OVER
MALE	55.8	58.8	59.6	56.3	57.2	60.2	51.8	61.2	61.4*
FEMALE	64.8	64.0	66.4	63.0	64.3	65.3	62.1	61.7	66.8*
TOTAL	58.5	59.8	62.1	58.5	60.0	61.5	55.9	59.4	62.9

* Life expectancy of the general population born in 1900:
Males. 48.2 years
Females. 51.1 "

of parental age is independent of sex differences and expresses itself in the lifespans of both sons and daughters, irrespective of whether the ages reached at death by the parents are considered individually or together. The comparatively highest mean age levels were attained by twin index sibships when an age of 70 years and over was reached by the mother alone, or by the father alone, or by both parents. There is no evidence for the operation of a sex-linked factor in the inheritance of general longevity potentials.

Based on these findings, the effects of genetic factors in ageing may be summarized as follows:

Genetic variations in adjustment to ageing may arise from

gene-specific traits manifested before senescence, but susceptible to its overstraining effect; or from specific metabolic dysfunctions peculiar to the senium; or from graded differences in general health and survival values. Much additional research is needed in all three categories.

Since genetic phenomena are the cause of many individual differences in the degree of ageing, it is inadvisable to approach geriatric problems with the preconceived notion that the adjustive difficulties of the aged are more or less the same for all persons and thus conducive to management by stereotyped methods.

Biological factors advantageous to adjustment to ageing include healthy and longevous parents, the efficacious use of genetic potentialities for physical and mental health throughout life, and the establishment of adequate emotional adjustment before senescence.

REFERENCES

ADLERSBERG, D., PARETS, A. D., and BOAS, E. P. (1949). *J. Amer. med. Ass.*, **141**, 246.

ALEXANDER, F. (1956). *Amer. J. Psychiat.*, **112**, 692.

BOGOMOLETS, A. A. (1938). Proc. Conf. on the Source of Senility and the Prophylaxis of premature Ageing. *Acad. Sci., Kiev.*

BUCHWALD, N. A. (1957). *Proc. IX Annu. Meet. Amer. Soc. hum. Genet.*, in press.

CRESSERI, A. (1948). *Boll. Soc. ital. Biol. sper.*, **24**, 200.

DUBLIN, L., LOTKA, A., and SPIEGELMAN, M. (1953). Length of Life. New York: Ronald.

ESSEN-MÖLLER, E. (1946). *Acta psychiat., Kbh.*, **21**, 233.

FRIEDMAN, M., ROSENMAN, R. H., and BYERS, S. O. (1955). *J. Geront.*, **10**, 60.

GAKKEL, L. B., and ZININA, N. V. (1953). *Sechenov J. Physiol.*, **39**, 533.

GIANFERRARI, L. (1954). *In* Genetica Medica, ed. L. Gedda. Rome: Gregorio Mendel Institute.

GLASS, B. (1955). *Nat. Acad. Sci. Publ.*, **338**, 7.

JACOB, H., PYRKOSCH, W., and STRUBE, H. (1950). *Arch. Psychiat. Nervenkr.*, **184**, 653.

JALAVISTO, E. (1951). *Ann. Med. intern. Fenn.*, **40**, 263.

KALLMANN, F. J. (1952). *J. Hered.*, **43**, 89.

KALLMANN, F. J. (1953). Heredity in Health and Mental Disorder. New York: Norton.

KALLMANN, F. J. (1956*a*). *In* Mental Disorders in Later Life, ed. O. J. Kaplan. Stanford: Stanford University Press.

KALLMANN, F. J. (1956*b*). *J. chron. Dis.*, **4**, 140.

KALLMANN, F. J., ASCHNER, B. M., and FALEK, A. (1956). *In* Novan't Anni delle Leggi Mendeliane, ed. L. Gedda. Rome: Gregorio Mendel Institute.

KALLMANN, F. J., FEINGOLD, L., and BONDY, E. (1951). *Amer. J. hum. Genet.*, **3**, 65.

KALLMANN, F. J., and SANDER, G. (1948). *J. Hered.*, **39**, 349.

KLÖPFER, H. W. (1957). *Proc. IX Annu. Meet. Amer. Soc. hum. Genet.*, in press.

MALAMUD, N., and WAGGONER, R. W. (1943). *Arch. Neurol. Psychiat., Chicago*, **50**, 288.

MCFARLAND, R. A. (1956). *Bull. N.Y. Acad. Med.*, **31**, 14.

MEGGENDORFER, F. (1939). *In* Handbuch der Erbbiologie des Menschen, ed. G. Just. Vol. V/2. Berlin: Springer.

PEARL, R., and PEARL, R. DEWITT (1934). The Ancestry of the Long Lived. Baltimore: The Johns Hopkins Press.

SJÖGREN, T., SJÖGREN, H., and LINDGREN, A. (1948). *Acta psychiat., Kbh.*, Suppl. 82, p. 1. Copenhagen: Munksgaard.

SØBYE, P. (1948). Opera ex Domo biol. hum. Hered., Vol. 16. Copenhagen: Munksgaard.

VERSCHUER, O. v. (1954). Wirksame Faktoren im Leben des Menschen. Wiesbaden: Steiner.

WILKINSON, C. F. (1950). *Bull. N.Y. Acad. Med.*, **26**, 671.

DISCUSSION

Lewis: Prof. Kallmann, how far do you think the senile and pre-senile psychoses necessarily contain something that is identical with the process of deterioration which occurs in normal ageing? These conditions, although commonly associated with old age, can occur at much younger ages, down to 27 or 28 in rare instances, and I would have thought it possible that morbid processes which precede the appearance of the disease may have been present for very many years in both; the senile psychoses and the pre-senile psychoses seen in Alzheimer's and Pick's disease may have no more essential connection with the process of ageing than, say, Huntington's chorea has.

Kallmann: Huntington's chorea has been compared with the pathology observed in an early ageing process. I would not be able to offer an expert opinion on that. However, whatever is found in Alzheimer's disease (especially in Alzheimer's, not so much in Pick's disease) is so similar to the signs of ordinary ageing processes after the age of 80, especially when it comes to senile plaques and so on, that it would be difficult to think in terms of two entirely different entities. In some families with Alzheimer's disease, various members in successive generations have been found to be able to reach a very old age (80, 90, some have reached the age of 98), except for those who show the symptomatology of Alzheimer's disease. The question is complicated by the fact that some family members don't show Alzheimer's disease. At least histopathologically their brains look like Pick's disease, so that it can

be assumed that Pick's and Alzheimer's diseases may occur in the same family; that is, in a family that ordinarily has the capacity for very long life. It is probable, therefore, that a major mutation has occurred in a gene that ordinarily has a normal function in a longevous person.

Psychopathological symptoms as such don't mean much unless they are related to chronological age and general metabolic changes. They are known to differ from one person to another, although toward the end of their lives all human beings are apt to be maladjusted.

Lewis: The difficulty in using the neuropathological criterion is that the brains of old people who showed no psychological evidence of deterioration are sometimes indistinguishable from those of old people who had considerable dementia.

Kallmann: Yes, but one does not know the extent of cerebrovascular changes which differentiate them. It may be a greater or lesser supply of oxygen and other things that differentiate persons, rather than differences in the structure of the brain itself. Certainly one should be able to identify a biochemical deficiency factor so clearly defined as in Pick's or in Alzheimer's disease. It is almost unbelievable that we still don't know what this gene does and what kind of a defective state it produces. As long as we don't know that, it is not surprising that even ordinary ageing processes are not sufficiently understood.

Olbrich: Would you say that the rate of ageing, the rate of decline, is genetically conditioned or controlled in your twin studies?

Kallmann: Yes, as far as variations in traits are concerned which are measurable by psychometric tests.

Bourne: Has any study been made of the inheritance of Simmond's disease?

Kallmann: I don't think so.

Danielli: Is it possible to generalize in any way about the deterioration of characteristics controlled by single genes, i.e. major genes, as compared with characteristics controlled by polygene sets?

Kallmann: In the case of a single-factor trait which is due to the effect of a single major mutant gene, pathological changes are associated with some very specific biochemical deficiency states. As a rule, however, the ageing process takes place in traits that are polygenically determined. I don't know of normal personality traits that one would ascribe to the effect of one specific gene. Only when one deals with the effect of one gene that can express itself as clearly as is true in Alzheimer's or Pick's disease, then the effect is pathological as far as ageing is concerned. In the last analysis, ageing must be thought of in terms of the individual ability or inability to utilize ordinary longevity potentials.

Danielli: That seems to me an extremely arbitrary definition of ageing! In that case, how would you account for a man who lives twenty years longer than he should do because he has lived in a favourable environment? Something very peculiar would have to happen in his genetic set-up.

Kallmann: I don't think it is possible. How could you prove that someone lived twenty years longer than his genes were potentially capable of sustaining?

Danielli: Well, the question really is: is the present-day span of life

actually determined by genes, or is the action of genes merely permissive? Generally speaking, if you die before 40, in many cases it is not because your genes have cut you off at 40 or earlier, it is because you had a nasty accident of some sort! Now why should the causes of death be any different when you come to the eighties? It seems to me to be an entirely arbitrary assumption on your part.

Kallmann: How arbitrary it may be, I would not know. I have talked in tentative terms. We can only report what happens to a population of over 2,000 twins who reached the age of 60. If you think that we have selected material, distinguished by the ability to reach the age of 60 (at least one member of the pair), this possibility I shall concede to you. Of course, we cannot study people at the age of 60 who died at an earlier age. That would be too much to expect. But twins who are 60 years old can be followed for the purpose of determining what happens to them. In such a material, certain intra-pair differences observed in one-egg pairs are significantly different from those obtained in two-egg pairs. If such evidence is not accepted as substantiating a genetic theory, it is difficult to investigate problems of this kind.

Gillman: Prof. Kallmann, you showed two coloured slides of unusual cells. The first picture seemed to show a brown pigment and the second had green granules or green diffuse staining. Was this stain for iron or for lipids?

Kallmann: For lipids.

Gillman: Is this the nile blue sulphate method? It is unusual to stain lipid blue, with the brown granule. Was this only lipid or was it a lipoprotein iron complex?

Kallmann: In the first coloured slide, the yellow granules represent lipochrome pigment. As the name indicates, we are dealing with a complex lipid in addition to a yellow pigment (carotin). In the second slide, the reddish granules represent a mixture of fat-stainable substances as revealed by the Sudan III method and Cajal's gold sublimate impregnation technique.

Gillman: We have found mental disturbances to be common in Africans with pellagra or with severe liver damage unassociated with frank pellagra. Close relations are known to exist between nicotinic acid and tryptophan metabolism in the canine equivalent of pellagra, while abnormal indole and skatol metabolism are common in human pellagrins. These latter derivatives of tryptophan are closely related to 5-hydroxytryptamine. Consequently we have been considering the possibility that the mental disturbances in Africans with pellagra or chronic liver damage may be due to some derangements in serotonin metabolism, resulting directly or indirectly from malnutrition.

One wonders, in the light of Prof. Kallmann's data, whether any investigation has been made on two aspects: (1) on the physiology and (2) on the pathology of the people studied by him; the first, from the point of view of the excretion in the urine of derivatives of 5-hydroxytryptamine and/or lysergic acid, resulting from disturbances in the intermediary metabolism of these substances; and, secondly, whether in his cases any studies have been conducted comparable with those of

Hess (1955, *Nature, Lond.*, **175**, 387) on the ground substance of the brain. It would seem, from the studies of Hess, that disturbances in ground substance mucopolysaccharide, in cerebral tissue in particular, may play an important part in regulating the activity of the neurones themselves. Have similar studies been made by you?

Kallmann: No, the brains presented came from patients who died in some of our rural mental hospitals, and only the brain material was received for study. I showed these slides largely to demonstrate differences between changes observed in Pick's atrophy and those observed in Alzheimer's atrophy. Apparently, one can distinguish the two conditions histopathologically, although the given cases were not studied clinically. Our theory is that there has to be some different metabolic disturbance at the roots of these cases, and it is regrettable that we still do not know the nature of the metabolic defect involved.

Gillman: It would seem that, particularly with modern staining and paper chromatographic methods, the study which I suggested would be reasonably easy to conduct and may be worthwhile.

Lewis: I think it might be more difficult than that, because since interest was aroused in serotonin and its derivatives, many people have been studying the body fluids of psychiatric patients for such products, and so far very little has come of it.

Gillman: Have any such studies been done in Alzheimer's and Pick's diseases?

Lewis: No.

Friedman: I take it, Prof. Kallmann, that the extension of the lifespan which has occurred in the last half-century would indicate that the genetic characteristic was permissive rather than obligatory?

Kallmann: One can talk only in terms of potentials, and different generations may make a different use of their potentialities. It is only those who do not die before the end of their biological lifespans, who can show how long they may live. On the whole, in one-egg pairs we find a relatively close correspondence between the ages reached and the causes of death, shown at death, even in those who have been separated for fifty or sixty years and have lived under different circumstances. In two-egg twins, even in those who have always lived together, we rarely find such a close correspondence. We record these data, we don't produce them experimentally.

Best: Histological changes in very old people are the same as those in Alzheimer's disease, therefore what is the distinction between the elderly people in whom you do not diagnose this condition and those younger people who have the disease?

Kallmann: Whatever the senile plaques are, they are an indication of some metabolic disturbance in the brain. Ordinarily, they are not found before the age of 80 or 85. In cases of Alzheimer's disease, however, they are seen in the brains of people dying in their forties or fifties.

Best: Does that mean that everybody over 85 has Alzheimer's disease?

Kallmann: No, everybody's brain may show plaques characteristic of senile processes, apparently as the result of metabolic disturbances which ordinarily occur only in persons who die late in life.

Lewis: The trouble is that no one except Gellerstedt in Uppsala has examined, as far as I know, a large series of the brains of normal old people who did not show dementia. Oscar Vogt made a study of a small series of brains of very intelligent old people who had died without deterioration; but of any representative series systematically examined it is only Gellerstedt's that is on record, so we are held up constantly by the lack of a norm.

Lorge: Frederic D. Zeman, who has studied a large number of brains, tells me that there is no relation between brain pathology and a previous behavioural pattern. I don't know anything about longevity, so the question is "What are you correlating with what?" You may get corresponding gross anatomical pictures and yet not know what the previous behavioural history really was.

Kallmann: Even if something is so gross and conspicuous as senile plaques, we do not yet know what it means in histological and biochemical terms.

Lewis: We had a family where several members of a sibship—two sisters and a brother—had died of a pre-senile dementia coming on at the age of 43–45. There were seven other sibs, and two of these (still under 50) were becoming forgetful and incompetent, and apathetic. We were able to get biopsy specimens of the brains of these two and they showed argyrophil plaques and other features found in Alzheimer's disease. This was an instance where the brain changes, taken in conjunction with the clinical findings, clinched the diagnosis during life.

Nicolaysen: Have any studies been made of the oxygen consumption of the brain under various circumstances, in view of the fact that under ordinary conditions the brain is responsible for about 20 per cent of the basal metabolism?

Lewis: Yes, there are studies on that.

Best: Are there studies on each particular disease?

Olbrich: Yes, Scheinberg, Kety and Fazekas in America have done estimations of the blood reaching the brain, the oxygen uptake and sugar uptake; and they have shown quite clearly that in correlation to the norm in these cases the blood supply to the brain is diminished per 100 g. of brain tissue. They found also that the sugar uptake and the oxygen uptake were diminished.

Prof. Kallmann, plaques and fibrillary changes of the Alzheimer type are found in people who have no senile dementia at all, and many senile dementia brains show no plaques and no fibrillary changes of the Alzheimer type, so I don't know whether we are justified in correlating the two things. You see plaques and you see fibrillary changes; and you say that is the brain of a senile dementia; you speak to the clinician or to the man who has known the person and find that there were no symptoms of senile dementia. How can you correlate morbid anatomy changes or histological findings to a mental state which does not correspond?

Kallmann: We know that the ability of a person to withstand the effect of cerebral damage varies, but the thought that something so gross as senile plaques has no meaning in relation to brain function is difficult to follow.

Olbrich: I don't say "no meaning," only the "ascribed meaning".

Kallmann: Even if there are concomitant changes in blood supply or blood sugar or some other essential function, the primary basis seems to be some very specific metabolic defect, I would think.

Olbrich: Do you assume that it is due to fat changes in the brain?

Kallmann: Yes, this may be a suitable working hypothesis.

Danielli: It seems to be difficult to evaluate the twins data that have been presented to us today, since there is a strong tendency for these twins to seek the same sort of environment. The object, presumably, of twin experiments is to say that you have a common genotype, a standard genotype, and that enables you to evaluate precisely the effect of the environmental factor. But this seems to me to be partly vitiated by the fact that the twins do apparently largely seek the same environment and have similar experiences. Now, in the one set of data that was presented, on monozygotic and dizygotic twins, the average deviation from the mean in expectation of life, or accomplishment of life, for the monozygotic was about 5–6 per cent of the total length of life; in the case of the dizygotic it was about 10–12 per cent. The question that I would raise is: did the experience of life of the monozygotic twin pairs vary by more than 4–6 per cent? Also, did the experience of life of the dizygotic twin pairs vary by more than 10–12 per cent? If what one is measuring is simply the result of the variation in experience of life, then the data on these twins are more or less meaningless.

Lorge: There are enough American data to suggest that even under highly diverse environmental conditions the similarity of the monozygotics is extremely consistent. My feeling is that the Kallmann data would certainly exemplify, for most of the cases, a homogeneity of environment, whether it is sought that way or whether it is conditioned; but in those instances where he has been absolutely able to separate out those who have had highly diverse environments, he has a striking homogeneity of results.

Danielli: That raises the point as to how far these environments really were so diverse. Take human beings living in a civilized society: it is extraordinarily difficult to give them really varied environments, and if they are twins they seek the same sort of environment. I suspect that people working on twins overestimate the amount of diversity; to succeed in getting 10 per cent diversity of environment is quite a big thing.

Lorge: I think either way they are a very important methodological issue. What do we mean by "similarity of environment"? This concept of environmental homogeneity requires a considerable amount of investigation.

Kallmann: It is the objective of twin studies to determine the mode of interaction of genetic and environmental influences, rather than "to evaluate precisely the effect of the environmental factor". The popular notion that the behaviour patterns of one-egg twins resemble each other chiefly because of unusual similarity in their early environments has yet to be substantiated. Actually, if it can be shown that one-egg twins "seek the same environment" more frequently than do sibs or two-egg twins, this piece of evidence would strengthen rather than weaken any correctly formulated genetic theory.

METHODOLOGICAL PROBLEMS IN THE STUDY OF CHANGES IN HUMAN PERFORMANCE WITH AGE

A. T. Welford

The Psychological Laboratory, Cambridge

It is relatively easy in principle to obtain information about changes of performance with age, but to gain an understanding of the nature and causes of these changes is a matter of unusual difficulty. This is due partly to problems inherent in all psychological research and partly to certain of these being intensified when age changes are studied. We shall not here be concerned with large-scale fact-finding investigations such as surveys of the numbers of men in different industries or on various grades of work. These have been used in the attempt to study human capacity in relation to age and, when carried on by personal enquiry rather than by circulated questionnaires, are of value as indicators of issues for more intensive study and as checks on theories formulated by such study. They are, however, ill-adapted to provide a thorough understanding of changes of performance with age leading to theoretical explanations or detailed solutions of practical problems—their use for these purposes is not impossible but tends to be extremely laborious and expensive. The reason is that surveys which are easy to make are so only because they deal with whole industries or factories or with classes of work such as skilled, semi-skilled and unskilled, whereas the differences of human demand are between individual operations, of which many making a variety of different demands are usually included in the larger groupings. We shall instead concentrate on the methods of more intensive studies consisting firstly of experiments, usually conducted in a laboratory, and secondly of studies of actual work in industry either by

means of factory records or by direct observation and measurement of industrial performance.

Four topics will especially be considered:

(1) The relative merits of these different sources of data.
(2) Problems arising from the complexity of the human "mechanism".
(3) Questions of motivation—are younger and older subjects equally willing to try their best?
(4) Certain other problems arising from the need to compare subjects of different ages or test the same individuals more than once.

(1) Experiments and Industrial Studies

Experiments are well recognized as probably the most powerful tool we possess for the study of human performance. They essentially use tasks specially constructed to bring out some particular aspect of performance, such as speed or accuracy of movement or ability to perform one or other type of intellectual operation. The task may take any of an almost infinite variety of forms from drawing lines on paper or sorting a pack of cards to simulations of high-grade skills such as flying an aircraft or driving a car which involve substantial and elaborate apparatus. Whether simple or complex they aim at obtaining a sample of the subject's performance under controlled conditions where a precise record can be taken for subsequent analysis.

One important limitation of experiments is that it is seldom possible to continue them for a long time—usually a few hours is the maximum and in most cases about half an hour is all that can reasonably be demanded of a subject. This gives rise to two opposing objections. The first is that if older people are less resistant to fatigue than younger, they might be able to maintain a performance for a short period at a level which would be impossible over a longer working spell, and the experiment would thus unduly favour older people.

Smith (1938), for instance, found in an experiment in which subjects were required to assemble nuts and bolts, that the lowering of performance between men in their thirties and fifties was somewhat greater for an eight-hour spell than it was over a period of half an hour. The second objection is that if older people are slower at learning an unfamiliar task, difficulty shown by them in performing an experiment might disappear with long-continued practice, and the experiment would thus give a falsely unfavourable impression of their capacities.

Both these objections tend to be exaggerated. What little we know about fatigue in older people suggests that mental fatigue effects may sometimes become *smaller* with age, at least until the sixties (e.g. Botwinick and Shock, 1952). As regards improvements with practice, these have been studied in a few laboratory experiments and appear, in some cases at least, to be in about the same proportion in all age groups, suggesting that absolute differences of performance might be reduced by long-continued practice but that relative differences would remain about the same (see, e.g., Brown in Welford, 1951, p. 64). However, our present knowledge bearing upon these problems is scanty and both would repay further study. Meanwhile the point would seem to be in principle valid that the observations made in brief experiments need to be checked against long-practised performances to sort out continuing differences of performance from changes of capacity to deal with short-duration and unfamiliar tasks.

These objections do not apply to studies of industrial work. Observation and measurement of work in industry cannot be so closely controlled as an experiment, but are usually more so than other everyday activities and thus provide tasks practised to an extent far beyond what is possible in the most protracted experiments, yet capable of quantitative study in terms of output and a number of other measures.

For the clearest results experimental and industrial studies need to be closely integrated together. Experiments need

verification from industrial investigations; these need guidance from experimental results and can in turn give rise to further experimental enquiry.

(2) Problems arising from the Complexity of the Human "Mechanism"

When we study the behaviour of a whole human being we are attempting, even more than in other biological studies, to understand the working of a mechanism of extreme complexity which for almost all practical purposes we cannot directly observe. We have normally to be content to deal with relationships between behaviour and external variables impinging upon the organism. As a result, inference bulks large in the building of hypotheses, and interpretations are easily upset by new facts. Psychological theory thus tends to be unstable, and, as older people often highlight certain aspects of performance not normally considered by the general run of human psychological work done almost exclusively on young people, the student of psychological changes with age frequently has to re-think parts of his theoretical background to provide a setting for his findings.

The most important result of this complexity, however, is that investigations, whether experimental or industrial, have to sort out the effects of the many different mechanisms, sensory, central and motor, contributing to a single final result. This means that in experiments and direct studies of industrial work it is often not sufficient to take a simple measure of performance such as amount achieved in a given time at a single task.

We need to expand our study in three ways: first, by measuring performance under conditions which are systematically varied to limit one of the constituent mechanisms only, varying, for example, either the display of information or the responding action required while keeping the remaining conditions the same.

Secondly, we need to take several different measures of a

subject's performance: a single score of overall achievement leaves out of account variations in the manner whereby this achievement was attained. Thus, one subject may have been deliberate and accurate, whereas another may have been quicker in his actions but have wasted so much time making errors that he has achieved no more than the one who was slower. We need to measure various component actions of the performance separately; ending up, ideally, with a double analysis both of what the subject has done in terms of accuracy, types of error, form, etc., of his actions, and also of the time he has spent over each.

Thirdly, all these scores need to be examined for variation during the course of the performance: thus one subject may start slowly and speed up while another maintains an even tempo throughout, and yet another proceed by a series of bursts of activity with intervening pauses.

Many important studies have been done without entering into this amount of detail, and indeed few have dealt with all three types together, but significant differences have been shown between age trends in closely similar tasks, in different constituent actions of the same task and in the serial course of performance, so that each type of detailed treatment should be considered and only rejected after making sure that important data are not likely to be thrown away.

The same considerations apply to industrial studies based on production records. Figures for overall output can often with advantage be supplemented by records of wasted material and faults, and by figures for changes in all these with variations in the precise nature of the job and over periods of time. This is not to say that significant use of production records cannot be made without this extra information, but that there are indications that it is in such features as continuity of activity rather than overall production that early signs of change with age at industrial work are to be found.

(3) Motivation

Two questions are often raised about whether older and younger people are equally willing to co-operate in ageing studies, and whether unwillingness by older people distorts the results. The first is that the subjects of almost any intensive study must for obvious human and practical reasons be volunteers, and it may well be that these are on average bolder and somewhat abler than their contemporaries. If so, and if the older subjects are, in fact, more highly selected in this respect, age trends in their performance might be unduly favourable. The second question is that experimental tasks are almost always artificial and may be regarded by older people as trivial and not worth serious effort, with the result that age trends in performance might tend to be less favourable than they ought.

With regard to the first question, most investigators have found difficulty in obtaining subjects in middle and old age. It is fairly easy to obtain men in their twenties, but in the thirties and over they become increasingly unwilling. Many plead lack of time, or raise other difficulties, or agree to come and then forget. These pleas are, of course, sometimes well justified, but it is quite clear that in most cases they are excuses and that the real reason for unwillingness is fear of being tested and in particular of doing badly and appearing foolish. They seem to know well the popular opinion that as one advances through middle age one's ability falls, and do not wish to have this demonstrated upon themselves.

It has certainly been our experience that although many older subjects are unwilling to be tested, once they consent they approach their task with every intention of putting forth their best efforts and are fully as well motivated as those younger. Indeed, if there is any difference of motivation it is on the side of the older subjects, and if their performance is in any way adversely affected by motivational factors it is not by under-motivation but by over-motivation leading to anxiety with the consequent danger of disorganization.

Older subjects are, however, sometimes unwilling to sustain their initial effort. The first instance noted among the experiments by the unit of which the writer was a member was in an extremely irritating, noisy task which required the subjects to make several long series of judgments with no knowledge of whether they were doing so correctly or not. It seems reasonable in this case to suppose that the older subjects found the task disappointingly trivial and unrewarding. Subsequent instances, however, seemed to be due to a different cause. They were all in very difficult experiments, and what appeared to happen was that the older subjects would try the task but then realize it was beyond them and give up. A clear example of this behaviour is contained in a series of experiments by Clay (1954, 1957) in which subjects had to arrange numbered counters on chequer-boards to add up simultaneously to marginal totals on the rows and columns. She found that with a 3×3 board the task was done willingly, and usually successfully, by all subjects from the twenties to the seventies, although the older were a little, but not significantly, slower. With a 5×5 board, however, striking age changes appeared. Time taken rose from the thirties through the forties and to the fifties but in the sixties and seventies suddenly dropped. There was, however, at these latter ages a substantial increase in the number of errors made and some subjects failed to complete the task. Results for a 6×6 board were similar but with the "break" appearing between the forties and fifties. It seems as if the subjects up to the age at which the "break" occurred could complete the task by taking extra time and were willing to do so, but that for the oldest subjects the task was virtually impossible. They came to realize this fairly quickly and gave up after a short time. They did, in fact, take the most sensible course open to them in the circumstances, but it is clear that their unwillingness to continue was essentially the result and not the cause of their poorer performance.

Between the two extremes of irritatingly trivial and impossibly difficult, older people seem to enjoy experiments

once they can be persuaded to try and, especially where they can see some results for their efforts, become thoroughly absorbed in the tasks.

Motivation in industry should, on the face of it, differ little with age since all the men or women studied are doing their daily work. There are, however, two suggestions frequently raised which would make this view too naive. Responsibilities for feeding, clothing and possibly educating children, which are normally high in the thirties and early forties, will, for most men, decline in the fifties and might lead a man to be content with lower piece-work earnings or to transfer from piece-work to day work somewhere about the age of fifty. A considerable number of men do in fact move from very arduous jobs in their early fifties (Richardson, 1953) and piece-rate earnings show some tendency to decline at about the same age, but in view of striking changes of performance in experiments which also occur at these ages it seems doubtful whether such attitudinal factors are mainly responsible for the industrial trends.

The second suggestion regarding motivation in industry is that tacit agreements sometimes exist in a shop or factory that younger employees will work at less than maximum rate in order to "give the old ones a chance" or to ensure that they will themselves be able to maintain the pace when they are older. Very little is known definitely about the effects of either of these factors.

(4) Problems arising from the need to compare subjects of different ages or to test the same individuals more than once

What is probably the most recalcitrant and all-pervading methodological problem for ageing studies derives from the fact that a human being carries his history with him. Each new situation is dealt with in terms brought from past experience and in its turn modifies the experience brought to bear on future situations. The organism is thus constantly changing;

its present state cannot be fully understood without some reference to past events and we can never assume that it is the same on two different occasions—indeed we can be sure it is not. For many purposes these variations may be unimportant, but they do, at least for certain tasks, carry three important implications. Firstly, part of the variation between people of different ages living at any one time will be due to the fact that they were brought up under different conditions, and for the present population these differences are profound. Secondly, any study or testing of an individual will itself affect his approach to subsequent situations so that a prolonged study cannot be wholly independent of the effects of its own earlier stages. Thirdly, differing experience and development serve to magnify the variations between individuals, and may grossly affect their capacities.

All this means that at the very least we must study groups of people and compare substantial numbers in order to assess age changes. We cannot base any valid inference about the average capacity of older people from the exceptionally good or bad performances of a few individuals or from the occasional striking old men and women held up as examples of what older people can do in industry, politics and other walks of life. We have to think in terms of average trends, yet even these may upon occasion be of doubtful significance. A common finding is that the performances of older and younger people, say of a group in the twenties and another in the sixties, show a clear change of average performance but with a much wider scatter in the older age group than in the younger—many of the older group are well within the range of the younger, others well outside. The problem in a case like this is to decide whether the older group is comprised of some individuals who have changed greatly and some who have not changed at all, or whether all have changed to some extent with amount of change following a non-linear function. Only in the latter case can we justifiably regard the arithmetic mean as a representative measure of the performance of the older group. In the former case we should have to substitute for the mean a

statement in terms of the proportion of subjects attaining or exceeding a given level of performance. Fortunately for ease of statement the use of the mean does seem to be justified in most instances.

Other implications of the rôle played in human performance by past experience concern problems of sampling and differ according to whether the studies are (*a*) *experimental* or *industrial*; (*b*) *cross-sectional*, examining groups of different ages in the population at one time, or *longitudinal* following a single group over a substantial period of time; and (*c*) aimed at obtaining *theoretical* insight, or at the solution of an immediate *practical* problem. Each of these classifications is independent of the others giving a somewhat unwieldy array of eight types. The independence of (*a*) and (*c*) may at first sight be surprising since experiments tend to be used in theoretical research and industrial studies in practical problems. We have already pointed out, however, that industrial studies can contribute significantly to theoretical issues and there is a growing tendency to use experiments as a means of furthering practical research.

The obvious difference between theoretical and practical studies as far as work on ageing goes is that the former are directed to an attempt to understand the nature of ageing, whereas the latter are concerned with problems such as employment or social welfare to which the ageing process may be an important but nevertheless ancillary consideration. The main methodological difference between the two is concerned with sampling for cross-sectional studies.

Cross-sectional researches with a practical aim need to be done with a strictly representative sample of the population to whom the practical issues relate. They might thus require a representative sample of the population of the country as a whole, or of a particular town or factory or occupational group. The sample should be of the present living population in each case with no consideration of whether death, disease, emigration, education or any other cause has had a selective effect on the older age groups. Refinements to this basic

requirement may be needed if, instead of dealing with a present problem, we are trying to anticipate one in the future. We might, for instance, need to control education to allow for changes over the years in schooling, or health to allow for changes in the treatment of disease, but the principle is still the same—we are attempting to predict a representative sample of a future population.

The essential requirement for a theoretical study is a sample in which older and younger people are comparable. This is much more difficult than a representative sample to obtain with human subjects because differential effects of death, disease, education, occupation and so on must be taken into account, and in a rapidly changing world parts of the economic, social, material and educational backgrounds of subjects will inevitably differ with age even among people of the same status in every other respect. The problems of obtaining comparable samples differ between experimental and industrial researches, and we will therefore deal with the two types separately.

Experimental Studies

The obvious procedure is to attempt to control the backgrounds of the subjects so that the different age groups will be equated in all important respects. Unfortunately, just what respects are important appears to differ from one task to another in a manner which, with present knowledge, is not entirely predictable. There is, however, some evidence upon three types of factor: (*a*) educational and occupational level, (*b*) occupational "skill" and (*c*) family relationship.

(*a*) *Level of education and occupation.* Several researches have shown that declines of performance with age are less among people of high educational or occupational level. Effects have been shown for problems involving "ingenuity", translating an artificial language, giving synonyms and antonyms, symbol-digit substitution, completing number series, giving meanings of words, giving analogies and solving arithmetic problems (Sward, 1945); recall of a passage of prose and

a "test of concentrated attention" (Pacaud, 1955). The tasks have in common that they involve fairly high-grade intellectual activity, judgment or the use of verbal or other symbols. With more straightforward sensory-motor tasks differences of performance with educational and occupational level are usually small (Pacaud, 1955).

These findings may be linked with the result, often obtained in experiments and mental tests, that the difference with age between the best performers shows less decline than between those whose performance is poorer. This result occurs with a wide variety of tasks such as the Matrices test and word meanings (Foulds and Raven, 1948); memory, both short- and long-term (Gilbert, 1941); and sensory-motor tests (Pacaud, 1955). It is clearly another facet of the tendency noted earlier for variation between individuals to increase with age. It could in some cases be an artefact in the sense that the task may not stretch the ablest individuals and may thus fail to show differences at the highest levels of ability. This cannot be the complete explanation, however, and there seems no doubt that age declines are less among the best performers at least in a relative if not always in an absolute sense.

(*b*) *Effects of occupational skills* can sometimes completely reverse a group age trend for a particular individual. For example in an experiment by Szafran (1951) in which hand actions had to be carried out blind, a subject aged 49 who had considerable experience in dark rooms was able to do the task far better than any other subject tested either younger or older. The connection between occupational skill and experimental performance in a case like this is clear and reasonably easy to guard against when making up groups of subjects. Aspects of skill and tendencies to action of occupational origin do, however, appear sometimes to "work loose" from their original settings and become generalized to performances seemingly remote from their originals. For instance, in an experiment where subjects were required to throw at a target (Szafran and Welford, 1949) an apparent slowing of performance with age turned out to be due to the inclusion of a large

proportion of servicemen in the youngest age range. Subsequent tests confirmed that men with recent army experience performed the task significantly faster than those who had been in civilian life throughout the second world war. It seems impossible to control such influences in advance without building up a systematic body of knowledge on the nature and extent of occupational "transfer" effects. Until this is done they must be reckoned as an unknown hazard for the experimenter.

(c) *Control of family relationship* by comparing different generations of the same family would seem to be an obviously desirable procedure in studies of ageing, yet it is one which is seldom used. Doubtless this is in part due to the difficulty that would be caused by the additional constraint in the selection of subjects, but it may in part also be due to the curious unwillingness of psychologists in the past to admit the rôle of hereditary factors in determining human capacity. Resemblances between parents and children have been demonstrated in a number of performances, such as intelligence tests (Jones, 1928). Family resemblances in behaviour have been shown to be in part the result of heredity and in part the result of similarity of environment.

For a large-scale study we should take care to see that the numbers in each age range are balanced for these and any other background factors suspected as being of importance. For a small-scale experiment aimed at establishing a qualitative rather than an exact quantitative result it is probably sufficient to concentrate on subjects from one particular background—say from a single factory or a university population. The inferences we can make will be restricted owing to the restricted range of subjects, but not seriously so. An alternative procedure which overcomes this limitation is to take two or more groups of widely different background in each age range—in effect to do the experiment twice on different types of subject. By this method we not only achieve a high degree of control but also throw light upon whether differences of background are in fact important for our experimental task.

Four other methods of a very different kind are sometimes useful for ensuring or checking the comparability of subjects in different age ranges.

(i) When we know, or have reason to believe, that the observed age trends should progress smoothly over several age groups we can assume, provided our experimental method has been rigorous, that any marked deviation from a smooth progression is due to an accident of sampling. What appears clearly to be a result of this kind is contained in an experiment by Weston (1949) on changes of visual acuity in middle age.

(ii) Some investigators, notably Thorndike and co-workers (1928) have given their subjects a preliminary task such as an intelligence test, and selected groups in different age ranges with equal means and scatters of test score. This is undoubtedly a powerful method of control, provided we know what the preliminary task measures. The inference from the experimental results is, of course, affected in the sense that any trend is relative to that which would be observed in results of the pre-test for an unselected population, but this, far from being a disadvantage opens the way to a rather precise method of assessing the relative magnitude of age trends for different types of human capacity.

(iii) Somewhat similar to this last method is one used by Szafran (see Welford, 1951), Kay (1954), Clay (1956) and others which consists of presenting the experimental task in two or more different forms and noting the difference of age trend between them. In all the cases cited one form has been found to give much less age trend than another and we can thus use it as a base line for a relative statement about the effect with age of the factor by which the one form of the task differs from the other. This method appears to be of wide and rather simple application. Its value will depend, however, upon the easiest form of the task not being so easy that every subject can do it equally well. The task and the method of scoring must be such that some individual variation can be shown if the similarity between the older and younger subjects' performances at the easiest form of the task is to be more than a trivial result. In practice this means that in, say, a

problem task not only correct achievement but also time taken and possibly scores indicating the manner in which the achievement was attained need to be taken.

(iv) The most radical method of overcoming the problem of comparability is to abandon the use of human subjects in favour of animals whose background and experience can be rigidly controlled. There is, of course, some hazard in using the results of animal experiments as an aid to the interpretation of human performance, but insofar as ageing is due to biological processes common to different species, animal studies would seem capable of supplying invaluable checks upon human research. We may note that experiments on animal behaviour in relation to age seem to show very much the same trends as human studies, and thus tend to confirm that environmental factors cannot by any means wholly account for changes of human performance with age.

Longitudinal Studies

The difficulties inherent in cross-sectional studies can be avoided by following a group of individuals over a substantial period of time. The longitudinal method does however have two difficulties of its own which severely limit its usefulness.

(*a*) The method essentially involves testing a subject's performance and retesting it once or more later in life. This requirement means that any age effects are inextricably mixed with any learning effects. These latter can be very marked. For example, Heim and Wallace (1949, 1950) have shown that substantial gains in score on an intelligence test are made when it is taken more than once even when the subjects have no knowledge of their success or failure on earlier occasions, and that these gains transfer to another intelligence test. Their results were, however, for tests given only one week apart and thus may not be an entirely valid objection to longitudinal studies using intelligence tests such as that of Owens (1953). The effects of a single experience can, however, without doubt be long lasting and we need to know more about them before we can be confident as to the meaning

of the results of longitudinal studies using material of the level of an intelligence test on more than one occasion.

With repetitive tasks such as sensory-motor skills this difficulty can be at least largely overcome by training the subject before each test until he has ceased to improve with practice. Unfortunately, however, this may take a long time and make the test unduly time-consuming.

With problem-solving and other tasks where "insight" can be gained the task cannot be used more than once and subsequent tests have to be done with similar, but different problems. The problems must either be equated for difficulty or must be presented to different subjects in different orders in a balanced design. Even with different problems, however, substantial learning effects in the way of tackling particular types of problem are likely to enter, so that the longitudinal method would seem seldom appropriate for the study of performance at this kind of task.

(*b*) The obvious objection to longitudinal studies that a very long wait is required if the study is to span a substantial part of a man's life, can be overcome in some cases by using animals. Where animals are unsuitable, it may be possible to resort to a mixture of the longitudinal and cross-sectional methods. A group of subjects covering a substantial age range is tested and the same group is tested again after a period of years. For some functions this period can be as short as five years, as with Weston's (1949) experiment already mentioned. For most tasks, retesting subjects at intervals over a period of ten to twenty years would be better. This method has been little used because although it avoids the necessity of extremely long waits it still requires, with preparation before the first test and analysis of results after the second, a term of years longer than any research team on ageing has yet been able to look forward to with confidence.

Industrial Studies

Problems of equating subjects for education, occupation and other background features do not usually arise in industrial

studies because these normally relate age and work done and so control them automatically. They have, however, their own problems of control which depend upon the type of industrial data used.

(*a*) Production records or studies of actual work done provide what are at first sight the most valuable and informative data on performance, but are subject to serious limitations. The most important of these is that a marked fall in productivity would be unlikely to be tolerated for long by either the employer or the man concerned, so that if productivity falls much as a man becomes older he is likely to move or be moved to another job. Older people left on the job will thus be unrepresentative of their age group, and inferences based on their performance will be unduly favourable to older people. Selection in the opposite direction by promotion will also occur, although probably less often, and may result in age trends appearing unduly unfavourable to older people. We cannot without further evidence be sure in any particular case that these two selective trends balance. Progressive selection of this kind is not overcome by a longitudinal study of men who have been on the same job for a long period of years, because the fact that they have been so may imply that they are a selected group whose performance has declined or risen less than that of others.

Studies of work done in industry, whether cross-sectional or longitudinal, are thus valid only if labour turnover and transfers to other jobs are negligibly small. Ideally they require also a substantial group of people well spread over the age scale and all doing exactly the same work, since what are minor variations of work from the industrial point of view may imply major differences in the psychological demands the work makes. This requirement is very seldom satisfied in industry but can be at least partly overcome by relating each man's performance to the average of men in a particular age range—say the thirties—or in the whole group who are doing the same work. Wackwitz (1946) has used such standard measures to compare one operation with another in

his extensive and important study of industrial workers in Holland.

(*b*) It can be argued that if productivity figures are invalidated by progressive selection in favour of the most able, the age distribution of the men remaining on the job should give an indication of whether capacity to do the operation remains high with advancing age or declines. The age distribution for a job may, of course, be affected by factors such as age policies in the factory concerned or the age structure of the local population, but comparing the age distribution of one job, or group of jobs, with another in the same factory would seem to be a possible method of obtaining data on capacity in relation to operation. The data is crude, lacking all the subtleties of detailed studies of actual work done, but is a better method to use than production figures when labour turnover is high.

Age distributions may, however, be misleading if, as often happens, there is a policy of recruiting young people in preference to older, because they will then be dependent upon the history of the job. If the job has expanded, the age distribution will have too many at the young end; if it has contracted, too many at the old. It will for the same reason also be affected by such factors as the availability of labour in the district and competition with other jobs. Even where such a recruitment policy is absent, it may be difficult to decide whether a shortage of older people on a job is due to their failing to maintain performance or to their finding difficulty in learning the job with consequent restriction of placement. Age distributions are, however, by far the easiest industrial data to obtain and would seem capable of giving valuable preliminary information for comparing one class of job with another provided due precautions are taken to ensure that the recruitment policy and history is similar for both. Work by Murrell, Griew and Tucker (1956) has provided a striking example of stability in differences of age distribution between jobs over a period of eight years in a situation where there was no ascertainable selective placement.

(*c*) The most reliable criterion of difficulty for older people at a job is that substantial numbers leave at a relatively early age. The reliability is clearly greater for men's work than for women's and comparisons between jobs in terms of age at which people leave must be made separately for women and men. People transferred within the factory to other jobs must be included as well as those who leave altogether. The reasons given for leaving or transferring should be regarded with caution. Clearly when people leave because of gross disease or move from the district for family reasons we can hardly regard their decision as being influenced by a growing difficulty with age at maintaining performance at their jobs. Reasons such as this are, however, likely to apply equally to all jobs and therefore not to affect comparisons between them. Other reasons such as disagreements with management, dissatisfaction and even, as Richardson (1953) has pointed out, many cases of sickness do seem to be associated with jobs which we should expect on other grounds to become a strain as people grew older. Indeed, it would seem that in the absence of strong indications to the contrary all moves from jobs compared should be counted regardless of reason. A possible exception to this is where people leave for promotion or a better paid job, and a more rigorous criterion would be therefore to count all moves to lower paid work for any reason other than serious incapacity due to disease or accident and genuine redundancy. Anyone looking at changes with age can, however, scarcely fail to wonder whether many moves to better paid work are away from jobs which would soon have become a strain if the move had not been made.

Two important research possibilities for the future in this field may be mentioned. The first is the detailed study of performance at jobs where substantial numbers leave relatively young to see whether early signs of strain can be detected in changing methods or manners of work. The second is to use the cohort technique of taking a group, or set of groups of different ages and following them over a number of years not only at one place of work but also if they leave to go elsewhere.

Conclusion on Methodological Problems

It seems clear that many of the methodological problems commonly supposed to attach to the study of human performance in relation to age are less serious than they are usually believed to be. Difficulties arising from the brevity of laboratory experiments can be met by studies of work in industry. Motivation among older subjects does not seem to be a serious problem. The bewildering complexity of human capacity can, at least to a considerable extent, be sorted out. Various background factors affecting the comparability of subjects of different ages can be controlled.

On the other hand, it seems equally clear that no one way of studying ageing is wholly free from methodological objections and that we can thus seldom, if ever, draw certain conclusions from a single experiment or industrial study. In view of this it appears wiser to carry out several small-scale studies using different methods rather than concentrate the whole research effort on a few large-scale investigations. Even a study using thousands of subjects recruited haphazardly would be at the mercy of possible sampling errors. The use of a number of small-scale studies each controlling different variables enables us to gather on our way a great deal of valuable information upon the effects of various background factors influencing age trends, and although giving a mass of data which it may often be difficult to integrate, holds the promise of the attainment of results having a greater degree of generality.

REFERENCES

BOTWINICK, J., and SHOCK, N. W. (1952). *J. Geront.*, **7**, 41.
CLAY, H. M. (1954). *Brit. J. Psychol.*, **45**, 7.
CLAY, H. M. (1956). *J. Geront.*, **11**, 318.
CLAY, H. M. (1957). *Gerontologia*, **1**, in press.
FOULDS, G., and RAVEN, J. C. (1948). *J. ment. Sci.*, **94**, 133.
GILBERT, J. C. (1941). *J. abn. soc. Psychol.*, **36**, 73.
HEIM, A. W., and WALLACE, J. G. (1949). *Quart. J. exp. Psychol.*, **1**, 151.
HEIM, A. W., and WALLACE, J. G. (1950). *Quart. J. exp. Psychol.*, **2**, 19.
JONES, H. E. (1928). *27th Yearb. nat. Soc. Study Educ.*

KAY, H. (1954). *Quart. J. exp. Psychol.*, **6**, 155.
MURRELL, K. F. H., GRIEW, S., and TUCKER, W. A. (1956). Unpublished report by the Unit for Research on Employment of Older Workers. University of Bristol.
OWENS, W. A. (1953). *Genet. Psychol. Monogr.*, **48**, 2.
PACAUD, S. (1955). *In* Old Age in the Modern World, p. 279. Edinburgh: Livingstone.
RICHARDSON, I. M. (1953). *Brit. J. industr. Med.*, **10**, 269.
SMITH, K. R. (1938). *J. appl. Psychol.*, **22**, 295.
SWARD, K. (1945). *Amer. J. Psychol.*, **58**, 443.
SZAFRAN, J. (1951). *Quart. J. exp. Psychol.*, **3**, 111.
SZAFRAN, J., and WELFORD, A. T. (1949). *Quart. J. exp. Psychol.*, **1**, 160.
THORNDIKE, E. L. *et al.* (1928). Adult Learning. New York: MacMillan.
WACKWITZ, J. D. (1946). Het Verband tusschen Arbeidsprestatie en Leeftijd. Delft: Waltman.
WELFORD, A. T. (1951). Skill and Age. Oxford University Press for The Nuffield Foundation.
WESTON, H. C. (1949). *Trans. Illum. Engng. Soc., London*, **14**, 281.

[Discussion of this paper was postponed until after the paper by Dr. Lorge.—EDS.]

METHODOLOGY OF THE STUDY OF INTELLIGENCE AND EMOTION IN AGEING*

IRVING LORGE

Teachers College, Columbia University, New York

As more and more information about ageing becomes available, it becomes clearer and clearer that the interpretation of the evidence depends upon the methodology utilized and upon the assumptions underlying it. The appraisal of cognitive functions and temperamental traits depends upon at least three major considerations: (1) the means for measuring and evaluating intellectual behaviour and emotion; (2) the sampling of the populations to be tested or studied; and (3) the methods for obtaining the measures of abilities and traits at different chronological, physiological or biological ages. These factors, of course, are not independent since the kinds of tests, the sorts of people and the successive chronologies interact.

Despite the fact that the major concern of this paper is with the measurement of intelligence and emotion in ageing, the first topic will be that of the different methodologies for obtaining data about traits, functions or processes as related to age. In the study of humans, it is difficult to keep separate the relative contributions of heredity, environment and maturation, whether it be growth from infancy or decline toward senescence. In studies of physiological or anatomical traits, the researcher may be able to maintain close control of the environment; not so in behavioural studies of animals or of humans. The appraisal of the contribution of heredity to the learning ability of dogs gives some indication of the complexities involved in maintaining a stable and controlled

* This paper is primarily concerned with aspects of methodology. The substantive findings have been reviewed recently (Lorge, 1956 *a* and *b*).

environment. In the studies of humans, however, environmental controls are impossible. The very circumstances under which humans live introduce factors making for variations in behaviour. Indeed, it has been suggested that the age of the parents may be a factor in producing a different environment for subgroups of children. Among humans, there must be a reciprocal relationship between the effect of adult behaviour on child behaviour (Riess, 1954; Scott and Marston, 1950). Additional sources of variability are introduced by sampling different subcultures within a geographic unit, or by sampling at the same time different cultures. The socio-economic environment of today must affect the behavioural growth, development and decline, differently from the setting of the Renaissance or the circumstances of the ancient Egyptian dynasties. Different cultures at different times differed in their ways of caring for the young and the old, of inducting their children into their society, of working to produce food and services and of maintaining the social values and mores. The very setting—social, economic, vocational—must have affected ageing not only in physiology but also in behaviour.

On account of the difficulty of controlling the environment of humans, most of the evidence about the relation of behaviour to age has been obtained by the "cross-sectional" method. Essentially, the cross-sectional approach, at its best, studies some trait (or series of traits) in more or less representative samples of specified age-groups in a population, where age usually means chronological age. The desideratum has been to select at each age a representative (and sufficiently large) sample of five-year-olds, ten-year-olds, etc. Galton's pioneer measurement of hearing, seeing, reaction time, sense of perpendicularity and judgment (Ruger and Stoesigger, 1927) and Jones and Conrad's (1933) evidence about the growth and decline of intelligence were made by the cross-sectional method. The basic assumption is that samples of successive age groups are equivalent except for the changes which age brings. The assumption, however, is not consonant with the facts, for it should be obvious that

sampling and evaluating living samples introduces a bias. The survivors of a cohort at any age must differ somewhat from those who succumbed to the vicissitudes of life (cf. Thorndike *et al.*, 1934). Equally significant, moreover, is the fact that the samples of differing age groups may have had different environment and experiences, e.g. socially, in the values and attitudes acquired; or educationally, in the amount and quality of schooling obtained; or, vocationally, in the duration and intensity of the work load carried.

The cross-sectional method, therefore, allows comparisons between measures of differing age groups, i.e., it provides estimates about age differences rather than evidence about age changes. The method used to provide evidence about changes brought about in the ageing process is the longitudinal or follow-up. Essentially, the longitudinal method, at its best, studies some trait or traits in the same sample of individuals at successive periods in their lives. The follow-up studies of the intellectually gifted (Terman *et al.*, 1947), of the vocational careers of adolescents (Thorndike *et al.*, 1934) and of the intelligence test performances of college students thirty years later (Owens, 1953) are typical longitudinal studies. The assumption basic to the method is that nature (in the sense of genetic constitution) is more adequately controlled since the same individual is being evaluated at different times during his life-span. It must be recognized, however, that any cohort, to some degree, may be a function of its own special environment. A cohort born in 1886 would have been brought up in circumstances differing significantly from that to which the cohort now being born will be exposed. To this degree, then, the longitudinal method, by the very homogeneity of environment for any cohort, has some limitations. The difficulties in maintaining effective liaison with all members of the cohort or its sample, of keeping and training a loyal and efficient research staff to do the testing, are not nearly so crucial as are the difficulties introduced by the impact of social, political, economic, or military events on a specific cohort over its life-span. Except for Owens' study of intelligence and Kallmann's

researches on the social and intellectual data of senescent twin pairs (Kallmann, Ferngold and Bondy, 1951), most longitudinal studies have been concerned with the evaluation of growth and development in the period from early infancy to early maturity. The primary inadequacy of follow-up studies has been its relative inflexibility to capitalize on the development of new techniques for the measurement of traits or processes. Were a new method discovered for assessing emotion or intelligence or blood chemistry, the researcher would not be able to obtain retrospective measures of the trait. Occasionally, the need for repetitive measurements in the same sample at different times is eliminated because the generalization was established by the cross-sectional method.

Obviously, the two methods are complementary. In the ageing process, it is recommended that both methods be used simultaneously to capitalize on the advantages of each. The proposed method is tentatively named as the combined overlapping method. Essentially, the method would involve the selection of representative samples of individuals at specified ages (as in the cross-sectional method) and to follow each of these samples for a number of years (as in the longitudinal method). There then would be a number of cohorts, each of which would provide data at any time of age-differences between cohorts, and, after a period of study, an appraisal of age changes within each cohort, as well. Moreover, the relative consistency of the results for different cohorts, reaching the same age, would supply evidence of the effects of selective elimination by death or accident. The introduction of newer techniques, furthermore, would be facilitated, since the technique could be applied to any cohort over its shorter run of years. The combined overlapping method thus could provide evidence about age differences and about age changes.

Any method may be made more valuable by the utilization of retrospective data, e.g. as Kallmann has done so successfully in the senescent twin study (cf. Goldfarb, 1955). Retrospective data, whether supplied by the individual under study or supplied by others (parents, teachers, physicians, etc.) can

greatly enhance the value of data collected by any method. In personality appraisal, retrospective evidence about the parents' values and attitudes may be clues for the sources of the underlying motivations in their offspring. The influences of early learning upon later learning may have profoundly affected not only the behaviour of the child as a child but also that child as a maturing, and mature, adult (cf. Hebb, 1949). The limitations of retrospective data must be recognized: such evidence may be somewhat less reliable than data collected more specifically and more objectively. Retrospective data may be in error to some degree depending upon the recency of the event, the frequency of its occurrence, or the tendency to schematicize the evidence according to some preconception about what should be reported.

The method for accumulating evidence, therefore, introduces some special problems in the interpretation of evidence. A second sort of methodological concern relates to the representativeness of the samples of the population of different ages in cross-sectional study, or of a cohort for a longitudinal study. Wesman (1955), indeed, indicated the scope of problems involved in obtaining a representative sampling of adults at different ages for standardizing an intelligence test. The persons to be tested must be selected to be representative of the population by sex, educational attainment, occupation, residence (urban-rural), geographic region, etc. In addition, the person so chosen must be willing to co-operate by taking the test, and the examiner must be the kind of person who can keep the willing subject sufficiently motivated to make him willing to continue for a fairly long time, an experience that may be occasionally frustrating.

Since the optimal resolution of these complex factors is achieved but rarely, it is obvious that cross-sectional, as well as longitudinal, studies suffer from some kind of bias in the sampling. In appraising the intelligence of a cohort (Terman *et al.*, 1947; Owens, 1953), the bias has been toward appraising the ability of either the more intelligent or the better-adjusted, or the ability of the institutionalized old or mentally

disturbed in homes and hospitals. Hence, a major difficulty in appraising the ageing population depends on the biases in the samples selected and co-operating in the enterprise, as well as the inevitable bias that death and accident bring.

Finally, the major issue rests upon the method used for appraisal of the trait or the ability or the emotional adjustment of the individual chosen for the sample. As a psychologist, I am all too aware of the fact that it is not possible to suggest a useful means for evaluating the emotional adjustment or personality of individuals, especially over the wide range of adult ages.

Whenever social adjustment is measured in terms of goodness or adequacy, it will correlate positively with measures of intelligence. It may be surmised, however, that the social adjustment or the social competence of adults will show diminished relationship with test intelligence with increasing age. Brody (1942) concluded that social adjustment or competence, as age increases, depends "more and more on the products of (test intelligence) and less and less on (test intelligence) itself." As a matter of fact, the amount of relationship will be a function of the overlap in the meanings of intelligence and social adjustment or competence. It is, however, the variation among these meanings that constitutes one of the major problems in the interpretation of the results in intelligence and in adjustment. The long controversy over the relative influence of nature and nurture in the development of intellectual performances of children and youths basically rests on the semantic confusion among the meanings of intelligence and among the methods for appraising intellectual ability.

Intellectual behaviour may be conceived as the capacity to make good, adequate and acceptable responses. Such intellectual behaviour is the resultant of the interaction among heredity viewed as the potentialities of the individual, environment viewed as the opportunities afforded the individual, and maturation viewed as the development and perhaps subsequent decline of function in the living organism.

The appraisal of intellectual behaviour, however, is not easy. Existing measures of intellectual ability suffer in content, arbitrariness of units, and ambiguity in significance. In content, the tasks are varied—words, numbers, space relations, information, pictures, and the like. Certainly, the sampling of these and of other tasks must be representative of the array of tasks to which a person must respond in the course of a lifetime. Binet and Simon (Binet, 1908) emphasized, in their selection of tasks, the kinds of problems and the variety of adequate performances that are related to a child's life and experience and validated the goodness of response against a criterion of school success. To a degree, then, these tasks represented the range of cultural acquisitions included in school curricula. For Binet and Simon, such a sampling of intellectual tasks was, and is, reasonable. In appraising adult intelligence, however, it may be more reasonable to sample tasks from the range of experience that adult life affords. Unfortunately, this has not been done. Most of the tests used to estimate intelligence of adults are variations of the same kind of tasks used to predict scholastic success of youths and children. While it is necessary to obtain information about the success of adults in such tasks, it is more necessary to obtain a representative sampling of tasks that will appraise adults in terms of a complex criterion of success in the intellectual requirements of vocations, leisure-time activities, care of the young, economic adjustment and the like. The criterion of ability to learn may have to be enlarged to cover areas other than success with school work.

The significance of measures of intelligence depends upon the criterion. Even in the child and youth range, the criterion for the intellectuality of performance is far from perfect. In the adult range, there must be an attempt to obtain an adequate criterion for intellectual behaviours, or conversely, to find out what the tests do measure. If the criterion for adult intelligence were known, then the tasks could be selected and weighted to maximize the relationship between the tasks and the criterion. Since it is not known, the best that can be done

is to estimate the relationship of the tasks (or the combination of them) to various criteria that sensible people would suggest. It is obvious that if speedy behaviour is the criterion, the tasks must include some measure of quickness. Other criteria, however, such as the highest level of thought a person can perform, require the consideration of tasks that will enable the appraisal of the level of thinking that the individual can do successfully. Neither criterion is sufficient; others must be considered. Nor should a test be rejected because it gives a fair estimate of one and not of others. The interpretation of mental test results depends upon their affiliations with criteria of success in adult life.

The scores derived from mental tests involving some sampling of tasks is arbitrary. The weights assigned to these tasks are either based on the judgment of experts, or on their relation to some outside criterion, usually another test of intelligence, or on a combination of judgment and validation. The sum, therefore, of the credits is arbitrary to the degree that the criterion does not fully reflect the variety of intellectual performances that adult life requires. In a sense, then, tasks involving speed, perception, thought and performance lead to an undifferentiated mixture predictive of some kind of average ability if these aspects are positively related. They may fail even in that, if, let us say speed and thought are negatively related. Furthermore, the nature of the score is such that the zero of whatever ability is being estimated is not known. Thus scores cannot be added, subtracted, divided, or multiplied meaningfully. Of course, such scores can be scaled to indicate the likelihood of that much ability in reference to some standard population. But again, the standard population must be clearly defined. Too frequently the standard (or norming) population is an *ad hoc* group that *would* take the test.

The appraisal of intellectual behaviour further suffers in another way. There is considerable uncertainty as to the process by which the person achieves a successful performance. To the same item, a child or a youth or an adult or a senile

person may give a correct (from the point of view of the key) response — but the child may have given a response on the basis of reasoning and the adult on the basis of rote memory. The credit is arbitrary but the significance of the credits depends upon the criterion and the degree to which the process is affiliated with that criterion.

It may be asserted that the nature of the criteria for adult intelligence, or intelligence in any age range, conditions the nature of the sampling of the tasks to be included in a test. Indeed, there may be some point in demonstrating that measures of intelligence for children are highly predictive of measures of intelligence in adults. To the degree that the relationship is high, the inference would be that intelligence is similar in the two groups, or that the criteria for it are the same. Nevertheless, the very variety of tasks in intelligence tests may be such as to reduce the relationship of measures of intelligence over time. For instance, Thorndike (1933), using the apparently homogeneous measure derived from the Stanford Revision of the Binet-Simon, shows that the relation between retest coefficients varies with the amount of time between retests. For repeated Binet examinations given within a single month the correlation is approximately 0·89, and for repeated examinations five years apart the correlation is approximately 0·70. Part of the difference in these reliability coefficients is undoubtedly due to errors of measurement. It is more likely, however, that a considerable part of the difference is attributable to inconsistency of what the test measures at different times.

Anderson (1939) has emphasized the apparent inconsistency of measures of intelligence derived from infant tests and from the Binet. He concluded "Infant tests, as at present constituted, measure very little, if at all, the function that is called 'intelligence' at later ages." Balinsky (1941), in his study of the factorial composition of the Wechsler-Bellevue Intelligence Scale for age groups 9, 12, 15, 25–29, 35–44, and 50–59 years, concluded that mental traits change and undergo reorganization over a span of years. This inconsistency

between mental test ability at different ages emphasizes the concepts of ambiguity of content and of process in the estimation of intellectual ability at different ages. Intelligent behaviour, however, may only be apparently inconsistent at successive ages to the degree that the variety of educational opportunities, of environmental stimulations, or of physiological changes in physical fitness, visual acuity, speed of reaction, or metabolism affect the scores.

The opportunities involved in formal education influence the test results of adults in a large variety of intellectual items. When it is recognized that "highest grade reached" is an undifferentiated mixture of quantity and quality, the conclusion is somewhat strengthened. Highest grade reached credits each year of formal instruction — academic, vocational, or leisure time — regardless of the quality of the instruction. It is significant historically that the Committee on the Psychological Examination of Recruits in 1917 (Yerkes, 1921) set among their criteria for a group test of intelligence, non-dependence upon schooling, but that the empirical results between the group test (predecessor to Army Alpha) and reported length of schooling was significantly correlated.

Many of the studies of cross-sections of the population, therefore, must consider the influence of differential schooling in relation to variations among age groups. If the older individuals have had less schooling, i.e. if the amount of schooling is negatively related to age, some of the reported relations of intelligence with age may be reflecting differences in educational opportunity. Despite this limitation, it is significant that mental ability, appraised by means of vocabulary tests, seems to be well maintained throughout life.

The relatively greater decline in performance tests as compared with vocabulary has been responsible for the invention of the notion of intellectual deterioration. Babcock (1930), utilizing the hypothesis based on one of Jost's laws that "the oldest learning is last to be lost" developed a test whereby intellectual level is estimated from vocabulary and compared the performances on other tests with this level. It must be

recognized that since vocabulary reflects schooling, there will be an overestimation of level for persons with more than average schooling and hence a corresponding overestimation of the deterioration. This overestimation will be most noticeable if the performance items show relatively lower correlation with schooling than do vocabulary tests.

Perhaps a more significant find with respect to differences of younger and older adults in mental performances is given by Goldstein (Goldstein and Scheerer, 1941). He suggested a difference in attitude (or process) toward intellectual tasks. Older adults approach intellectual tasks with more of a concrete attitude than do younger adults. Younger adults tend to show more of an abstract attitude. To the degree that concrete attitude is related to crystallization of approach, and to the degree that abstract attitude is related to fluid approach, these results of Goldstein should influence the interpretation of intelligence test scores.

Goldstein's dichotomy between fluidity and crystallization or between concrete and abstract suggests that ways of approaching intellectual tasks may be related to personality. It is regrettable that tests of emotion and personal adjustment are as yet far from adequate, especially for long-term follow-up and large-scale cross-sectional study. Most of the information currently available comes from data about acute or chronic emotional disturbances which a psychiatrist or a clinical psychologist can recognize. Over the years, increased sensitivity to emotional behaviour has shifted the referent for emotional disturbance, but the results, unless related to retrospective anecdotal material, cannot be used except suggestively for estimating the changes in adjustment in ageing or differences between age groups.

The usual measures derive from inventories of reported feelings and behaviours. The famous Woodworth Schedule asked respondents to indicate for themselves answers to questions such as "Are you afraid of high places?", "Do you faint at the sight of blood?", etc. Such schedules have been used widely especially in military settings but they have not

been validated against an appropriate criterion for civilian adjustment over a wide age range.

Methodologically, there is a current need to develop more appropriate and more adequate measures of intelligence and of personality for long-term study over time. The intelligence tests, used in research, differ in their factor composition. Thurstone (1955), for instance, has produced evidence that his seven "primary abilities" develop at different rates in the age range from birth to maturity. If intelligence is multi-faceted, the problem is made much more difficult. Nevertheless, reasonable estimates of the primary component can be made. For a full understanding of cognitive growth and development, however, a variety of different tests appraising speed, "personal tempo", memory, space relation, vocabulary, number competence, etc. must be validated.

The greatest difficulty, currently, lies in the development of useful measures of personality and personal adjustment. Although the tests based on Rorschach's (1948) Psychodiagnostik open up an important area for test development, it will be many years before an adequate series of such tests will be available. The response of an individual to ambiguous or unstructural stimuli suggests that perception is related to personality, intelligence and experience. The isolation of components will be a long-term task.

The continuous appraisal of the ageing process thus depends primarily on the development of more and better psychological tests for cognitive processes, for personal adjustment and for psychomotor behaviour. The very limitations in tests and measurements for the psychological fields is a stronger reason for the use of the combined overlapping method in the collection of evidence for it is the method that can be most flexibly used when new and better tests become available.

REFERENCES

ANDERSON, J. L. (1939). *J. Psychol.*, 8, 351.
BABCOCK, H. (1930). *Arch. Psychol.*, *N.Y.*, no. 117.
BALINSKY, B. (1941). *Genet. Psychol. Monogr.*, 23, 191.

BINET, A. (1908). *Année psychol.*, **14**, 1.
BRODY, M. B. (1942). *Brit. J. med. Psychol.*, **19**, 215.
GOLDFARB, N. (1955). Longitudinal Statistics—Their Quality and Application, New York University.
GOLDSTEIN, K., and SCHEERER, M. (1941). *Psychol. Monogr.*, **53**, no. 239.
HEBB, D. O. (1949). The Organization of Behavior. New York: Wiley.
JONES, H. E., and CONRAD, H. S. (1933). *Genet. Psychol. Monogr.*, **13**, 223.
KALLMANN, F. J., FERNGOLD, L., and BONDY, E. (1951). *Amer. J. hum. Genet.*, **3**, 65.
LORGE, I. (1956*a*). *Annu. Rev. Psychol.*, **7**, 549.
LORGE, I. (1956*b*). *J. chron. Dis.*, **4**, 131.
OWENS, W. A., JR. (1953). *Genet. Psychol. Monogr.*, **48**, 3.
RIESS, B. F. (1954). *Ann. N.Y. Acad. Sci.*, **57**, 606.
RORSCHACH, H. (1948). Psychodiagnostik. Bern: Huber.
RUGER, H. A., and STOESIGGER, B. (1927). *Ann. Eugen., Lond.*, **2**, 76.
SCOTT, J. P., and MARSTON, M. (1950). *J. genet. Psychol.*, **77**, 25.
TERMAN, L. M., *et al.* (1947). The Gifted Child Grows Up. Stanford University Press.
THORNDIKE, R. L. (1933). *J. educ. Psychol.*, **24**, 543.
THORNDIKE, E. L., *et al.* (1934). Prediction of Vocational Success. Commonwealth Fund, New York.
THURSTONE, L. L. (1955). The Psychometric Laboratory. University of North Carolina, no. 14.
WESMAN, A. G. (1955). *J. Geront.*, **10**, 216.
YERKES, J. (1921). *Mem. Nat. Acad. Sci., N.Y.*, no. 15.

DISCUSSION

Best: Dr. Welford, is there any evidence that the younger people became bored during your experiments? Is the falling off due not to lack of ability but to the fact that they got tired of trying?

Welford: It is possible that the youngest subjects in a few experiments might have got a little bored, but that could not be said of the older, so that any decline with age could hardly have been due to boredom. Equally, however, it would be unsafe to argue that where an improvement occurs with age, there are certain capacities which mature in middle life rather than early on.

Tunbridge: In reference to Kay's (1954) work don't you think that the age difference could be very largely attributed to changes in eyesight? You are testing alignment and your change begins to become apparent at ages when you would get marked changes in vision and in the power to accommodate. Surely you ought to split the sample on their visual acuity and powers of adaptation? A similar problem has arisen in testing for dark adaptation in the determination of vitamin A deficiency. It was found that the tests were quite unreliable after the age of 40, although they were reliable in the younger age groups.

Welford: The task was in no way visually exacting. The numbers were the most difficult part of the display to see but they were about an inch high and viewed at not more than four feet, so they were really quite large.

Tunbridge: But it is an alignment factor and a question of focussing; the further the distance the greater the difficulty in accommodating. It is surprising what a difference a small distance would make to an older person.

Welford: Kay tried the alignment task without the numbers and this, in fact, yielded very little change with age. We can say that given the task of alignment only or going through the motions with the numbers only, you get very small change with age; given the two things combined you get a very large one.

Lorge: It is a double problem. Not only is it a visual task, but also it is the process of utilizing the informations, i.e. the transfers are important. Here one may make a point: when we train young people to learn codes, and we train them on meaningful material in which the expectations are already built into the individual, the learning is quite different from when they have to learn cryptic codes where no expectations are set up or used. What may happen is that the expectations already established by previous learning in the adult are so predominant that he expects anyone to be sensible enough to have the light related to the stimulus, that these expectations are overlearned, and thus he may have great difficulty in unlearning a learning. One of the findings in our work in learning is that it is much harder to break previously acquired habits than to learn rather new ones. For instance, when we tried to teach Russian, we used a group ranging from 20 to 70 years. If we organize the instruction so that all have success on every learning occasion, there is little or no difference in the amount of Russian acquired over time, i.e. over a 2- or 3-month period. The reason is that they have no prior expectations with a new language, a new symbol, a new anything. But when we try to teach Pitman shorthand, the adults do have tremendous difficulty because of their earlier over-learning of phonetic habits; they cannot break them, e.g. they try to write a *ph* symbol instead of an *f* symbol.

Olbrich: I have one fundamental criticism of your sampling of populations, namely, have you any health inventory of your subjects, i.e. have you tested the haemoglobins, blood pressure and blood urea, etc.? You may have a person with a haemoglobin of 70 or even 50, living quite happily, functioning perfectly, looking quite intelligent, and when it comes to the test he will give a result different from that given by a subject with a haemoglobin of 100. This applies to factory workers and also applies to an elderly population, even to young schoolchildren and, therefore, it is a big question: what factors play an environmental rôle? There is the influence of haemoglobin or, in the older group, of a high blood urea. You may have a university professor who has a quite intelligent response and his blood urea is up to 120, so these tests are not valid.

Lorge: Your criticism is extraordinarily valid. We have had, as

another issue, the estimation of health in individuals. We have tried to study a representative community in New York State. We wanted to get a relation between responses to questions about physical and mental symptoms and psychological behaviours and health status. We enlisted the co-operation of some 30 physicians. All agreed that they can recognize ill-health but none was willing to report a measure of the adequacy of the health status of the individual being appraised. Your point, however, is well made. Wetzel, in the United States, demonstrated that certain measures of health are related fundamentally to the school success of children, and that the child's health can be estimated, in part, by the fact that he is not achieving adequately in school; this is recognized as ill-health. On medical treatment, which may be nutritional or corrective in another way, the school performance does, in many instances, improve and the averages definitely do go up. The number of variables that must be kept under control in the appraisal of performance or of intelligence or of emotion are so complex that sometimes it is very courageous of a psychologist to attempt to do anything. After Prof. Danielli's contribution, I have a feeling that you people are in the same spot. Perhaps by the compounding of assays in the penumbra of shadowy ignorances, we may learn something.

Welford: I entirely agree, but I do think there is one point which is very often missed and that is that the type of sample required and what we need to control vary greatly according to the purpose of our study. If we have simply an applied aim, for instance, to further the employment of older people, we need to study the older people as we find them in the existing population; we need then a truly representative sample. We might perhaps complicate this by saying we need a representative sample of the population as it will be ten years hence. The sample would still be fundamentally of the same type. For a study of ageing, as such, we do not need a representative sample; we need one in which people of different ages are comparable. In a representative sample, death, disease and so forth all take their toll; but this does not affect the validity of the sample. In the comparable sample such factors are important and their effects must be controlled.

Friedman: I wonder whether there would be any validity in one additional approach to the two which Dr. Lorge has mentioned in his combined approach. The problem, it seems to me, is in part complicated by the fact that the testing method itself is a variable in the experimental arrangement. Could that be smoothed out by the ordinary biological procedure of selecting groups which were even on the test, i.e. in which the test performance was the same, and then working from that to a study, say, of ageing or other factors?

Lorge: That method has been utilized in some testing of the intellectual ability of adults at age 34, for whom scores on mental ability at age 14 were available. Individuals were equated for age-14 scores and measured again at age 34. The test results at age 34 were different depending upon the amount of education intervening between the fourteenth and thirty-fourth birthday. The greater the amount of schooling in the twenty-year interval, the greater the difference in the age-34 perform-

ances. However, the method has limitations. The limitations may be of importance, i.e. not only is there a limitation of repetitive testing, but also the kind of test appropriate to a 14-year-old does not allow enough room for growth or status as would the test appropriate to a 34-year-old. If we use a test that has unlimited ceiling so that a person can really reach its top limits at adult years, we may frustrate the child so that he gives up. Such a frustrating experience may be seen in Clay's data for old people; it is frequently seen in youngsters. They just will not try, and in our culture we get more "no trying" today than was true 30 years ago.

Danielli: Your attitude to motivation up to the moment has been rather negative. Now, what happens if you try to use it positively and give a reward that would be appreciated, e.g. getting the answer to the 64-dollar question might improve performance greatly; correspondingly, people might fill in a football-pool coupon much better than they would fill in the things in your assessment.

Lorge: In our own researches in the United States we have tried the influence of successive rewards, rewards in which the word was "right", or "you are doing very well", as opposed to "here is a penny for a successful pass, here is a nickel, here is a dime, and here is a quarter". The amount of learning, as a function of each of the different rewards, was approximately the same, with the 25 cents being somewhat less efficacious than the nickels and other rewards; the 25 cents disturbs people, it is too much! My general tendency is to generalize that the human is organized to react to confirmation. Anything he does that gives him a sense of adequacy and a sense of success is rewarding, whether it be a nickel or a penny or just a nice word from a responsible person.

Parkes: I think Dr. Welford is entirely right in saying that middle-aged people are not very co-operative, they know their reactions are failing a little and they are reluctant to have that fact dragged into the light of day and analysed. That is one very real explanation of the difficulty of getting subjects. But there is another possibility. As we get older our time becomes much more valuable to us for the simple reason that there is less of it to come and, therefore, we concentrate on the things we are interested in and, of course, playing ball with experimental psychologists may not have become one of our interests. I also agree with Dr. Welford that when a member of the older age group does get onto a job, he does his very best and also he may, as Prof. Danielli was suggesting, appreciate and respond more to a possible reward.

Lewis: It has been shown by Yerkes and Dodson that if people go at certain types of job too keenly and energetically, they do worse than if they went more quietly at it, as older people tend to. Surely Dr. Welford's point about motivation in his groups derives from the fact that he was dealing with a highly biased group selected for willingness; they were all volunteers. If he had a truly representative sample of any kind, he would not be able to count on that any more than one can, for instance, in institutional groups who are like a captive audience. You are dealing here with the temperamental and emotional changes as age advances which will greatly influence people's willingness, as well as their capacity, to participate in tests.

Dr. Lorge, you emphasized the difficulty in finding a yardstick for intellectual capacity in older ages since you don't have schooling as the criterion. You implied that such things as social intelligence must be taken into account, also the intelligence shown in one's job or in one's use of leisure. This is the sort of problem that has arisen in several other contexts and that has been dealt with effectively by starting with criterion groups made up of people who show to an extreme degree the qualities which you are interested in, and then you use factorial analysis or similar statistical procedure, for determining what tests will serve your purpose best and yield a measure of the dimension or dimensions in question. Here we are dealing with several factors which contribute to the general notion of intelligence and we need statistical devices to overcome at various ages the difficulty of not having a satisfactory single criterion.

Lorge: I have done factorial analysis on cross-sectional samples, using Balinsky's data on the Wechsler. The percentage contribution of the first principal component changes with age. In the age-9 group, the first factor accounts for about 44 per cent of the total variance; in the 12-year group, 41 per cent; in the 15-year group, 31 per cent; in the 25- to 29-year group, 27 per cent; in the 35- to 44-year group, 38 per cent; and in the 50- to 59-year group, 50 per cent. This is the complication that is introduced, so that while your suggestion, Prof. Lewis, is meritorious, what we have here is that the principal component is based on a very limited array of tests, and what we would have needed is of the order of 40, rather than of 10, different kinds of tests. Jones and Conrad assert that a youngster gets 25 per cent of his score from both the vocabulary and information test, but an adult gets approximately 40 per cent of his total performance from those two tests. It is the same thing, the principal component is variable and it seems to be smallest around 30 years, which is the age at which reaction time begins to affect performance.

The other issue is whether we have a cultural phenomenon related to the concepts of accuracy: the data on older people seem to indicate that accuracy is something that these people work for and work at. They like to be accurate, they are not always so. In our work with the Western Electric Company in the United States during the war, they re-hired—at my suggestion—a number of people who had been retired. The total performance over the year in terms of useable products was greater for the re-hired people than for the regular younger staff. The reason was that the youngsters did not mind taking a day off to go fishing, whereas none of the oldsters did that. The only occasion on which they would take time off was when they were really ill. It seems as if the older people have devotion to task, commitment to accuracy and maintenance of material. Now is that cultural? Will our next generation be less that way? I cannot say, but it is an important phenomenon. We have to be aware of it; 50 years hence we may have a different kind of behaviour.

Welford: My comment to Prof. Lewis is that I think the possible inequalities of motivation in subjects of different ages are of a kind such that one has to limit the inference that one makes from any experimental

results. One cannot, at present, in most cases be confident about precise quantitative changes in performance with age; what one can usually say with justification in a properly designed and controlled study is, "If I find any change, any decline in the inevitably biased sample that I have got, then *a fortiori* there would be a decline in a representative or truly comparable sample."

Verzár-McDougall: Dr. Welford, how far does memory come into your tests? Our animal experiments are very crude in comparison with your human experiments, but we have the advantage in using animals (in our case, rats) that we can test groups of different ages and make longitudinal experiments. We have, however, a great problem about how to produce a motivation that is comparable in the young and the old individual. In longitudinal tests, we have a certain safeguard in that we test rats—young, middle-aged and old—up to five times during their lifespan on the same multiple maze, and we find that some of the very old rats tested for the fifth time during their life show perfect memory of the maze. The motivation is hunger, in all cases. They have an interval of two months and are then brought to the maze again for a memory test, and we may suppose that these older rats, since they show perfect recall, are as well-motivated as the young. An interesting point in our experiments with the older animals is that while some old rats, up to the very day of their death, showed perfect recall of the maze (which is perhaps relevant to Dr. Olbrich's point about the possible pathological condition), others which had also successfully learned it several times in their life suddenly showed, at the age of about two years, complete loss of memory. Although they appeared to be perfectly well motivated (insofar as we timed their running through the maze and their time was comparable to that of younger rats), these old rats were now completely incapable of recalling or relearning the known task.

Welford: There are, I think, really two kinds of memory loss which may come with age. One is of long-term memories which have been acquired a long time ago. The other is a much shorter term and more ephemeral memory. Mrs. Verzár-McDougall's experiments deal mainly with the first kind. Our experiments, when they involve memory at all, are concerned with the second kind. This enters into many problem-solving tasks, and lowering of capacity of short-term retention is probably one of the main causes of decline with age at performance at this kind of task.

GENERAL DISCUSSION

Best: Prof. Gillman, what is your conception of the cause of liver disease in South Africa? I realize the breadth of this question.

Gillman: One can find no so-called "specific" cause such as bacterial or parasitic infections. Since siderosis may commence between the ages of 15 and 16 years and seems to progress rapidly after the age of 20, at a time when nutritional failures—with frank clinical syndromes of pellagra and the like—occur, one suspects very strongly that long-term chronic malnutrition, so common among the African people, is an important aetiological factor.

It has been suggested by others that parasitic factors may be involved. Malaria is certainly not operative, either in Johannesburg or Durban. Helminthiasis and especially hepatic amoebiasis, both, occur in Durban and its environs. However, another study which is being conducted in our group by Dr. N. Lamont indicates that amoebic liver abscess is frequently superimposed upon a previously damaged and siderotic liver. Also, Dr. R. Elsdon-Dew who has for many years worked on amoebiasis in Durban, has provided considerable evidence (1949, *Amer. J. trop. Med.*, **29**, 337) indicating that the susceptibility to *Amoeba histolytica* infection is a function of the nutritional state of the individual. Similar evidence has been obtained in experimental animals by workers in Washington (Taylor, D. J. (1950). *J. Parasit.*, **36**, sect. 2, 21). So, it would seem that the high incidence of liver disease in the African in the Union is primarily due to long-term chronic under- or mal-nutrition and certainly not to acute deficiencies of one or other known nutritional component.

Bourne: The pictures that you showed of the deposition of iron in the liver, in particular, are strikingly reminiscent of the accumulation of iron which you get in animals with cobalt deficiency, a fact that would support your suggestion that it is nutritional in origin, except that I find it a little difficult to believe that there is a significant cobalt deficiency in these people.

Gillman: From what I know of the literature on cobalt deficiency (which I believe is mainly Australian in origin) there is usually a profound associated anaemia and the iron seems to be derived from haemoglobin. In Durban, anaemias are much more common than they are in Johannesburg, but are nevertheless rarely profound, even in siderotics—the haemoglobin in these patients ranging from 10 to 12 g. per cent. Nor is there any evidence of haemolytic anaemia. In Johannesburg, however, there is no evidence of anaemia of any kind.

yet hepatic siderosis is still common. In Kenya and Uganda, however, severe anaemias are common (Foy, H., and Kondi, A. (1956). *Lancet*, **i**, 423) but siderosis is absent. The excessive iron in the liver and other tissues must, in the ultimate analysis, come from the diet. But the mechanism promoting the prolonged absorption, from the gut, of excessive iron is not known. As indicated already, during the earlier stages of siderosis, the iron is almost entirely confined to the hepatic epithelium, myocardial cells and other parenchymal cells, and very little is present in the reticuloendothelial system. This picture speaks against a haemolytic origin of the siderosis. In the presence of chronic malnutrition the apparently large quantities of iron reported to occur in the African's diet (Walker, A. R. P., and Arvidsson, U. B. (1953). *Trans. roy. Soc. trop. Med. Hyg.*, **47**, 536) may also play some rôle either in promoting or expediting this disease. However, I do not think that a high iron intake *alone*, especially in a "good" diet or well-nourished individual, can promote such extensive and severe siderosis as we so frequently encounter. The experimental work of Kinney and co-workers (Kinney, T. D., Kaufman, N., and Klavins, J. (1949). *J. exp. Med.*, **90**, 137, 147) supports this view. There is also experimental work indicating that the alimentary mucosal block to iron absorption is normally influenced, indirectly and in part, by the iron stores in the reticuloendothelial system as well as, perhaps, by some function(s) of the pancreas (Gillman, J., and Gillman, T. (1951). Perspectives in Human Malnutrition. New York: Grune & Stratton, Inc.). Bearing in mind the large quantities of iron in the connective tissues of our cases, there would seem to be some other forces promoting excessive iron absorption and retention. We suspect some nutritionally conditioned intracellular disorder as the primary lesion coupled, perhaps, with a high iron intake in a primarily maize diet and possibly, in addition, some associated (also nutritionally caused) pancreatic dysfunction (Gillman and Gillman, 1951, *loc. cit.*; Kinney, T. D., Kaufman, N., and Klavins, J. (1955). *J. exp. Med.*, **102**, 151).

Bourne: Yes, but the accumulation of tissue iron in these people is very similar to the accumulation of iron that takes place in cobalt deficiency.

Lorge: Has anyone made studies, medical or otherwise, of so-called "underdeveloped areas" which were Westernized suddenly? Puerto Rico, for instance, in less than 50 years, has extended its life expectancy to almost that of continental United States. Now, did anyone make collections of livers (or of any other organ) and did anyone subsequently collect livers, after such a great improvement had taken place in the nutritional status, to see whether there is any fundamental difference?

Gillman: This has been one of our main pleas. Those of us in South Africa, who have been attempting to get to grips with this problem, have tried to impress upon people the need for gathering the material before it is gone. This is something for which we have hitherto received very little assistance. One wants not only financial assistance, but help in terms of people to come and exploit the unique material in our country. Available evidence indicates that the reverse is happening in South Africa compared with that described by Dr. Lorge in Puerto Rico. There seems to have been a gross deterioration in the nutritional status of the African people during the last 50 years. The first cases of pellagra were reported during the Zulu rebellion at the end of the last century, and the first epidemic of pellagra occurred in the 1920's; so this deterioration seems to have supervened rather suddenly. The adoption by the African, in South Africa, of European customs and dietary habits is a relatively new thing. When I was a medical student, not so long ago, three-quarters of the Africans attending an Outpatient Department were blanketed. With the coming of World War II, relatively few Africans wore European clothes; but there has been such rapid industrialization in South Africa during the last 15 years that by the end of World War II it was rare to see an African attending an Outpatient Department in a blanket. This gives some indication of the speed with which this change is occurring among the Union Africans. Associated with this is a rapid deterioration in the agricultural value of the land available to these people in various parts of the Union. This is another basic, socio-economic aspect of the overall problem in my country.

Olbrich: If you compare your agricultural population, still blanketed, to the industrial population, you already have the answer.

Gillman: We are trying to get something organized along these lines. So far, we have carried out only a relatively limited survey in a completely different rural population of African fishing folk, near Lourenço Marques in Portuguese East Africa. We have taken liver biopsies from some 50 or 60 adult volunteers. But this group were eating a particular kind of diet, different from that consumed by either the rural or urban populations in the Union. It was a fishing community whose main article of diet was fish. Thus their diet contained quite large quantities of proteins, vitamins A, D and B complex. All the information thus far available from nutritional surveys among urban and rural Union Africans indicates that malnutrition and pellagra are common throughout our country. We hope in the near future to acquire precise data concerning the incidence of liver disease in rural and urban Africans in the Union.

Zilli: I would like to comment on what Dr. Landowne and Prof.

Tunbridge said about normal people. I agree with them that after a certain age (let us say 60 years) it is impossible to consider normal people. We arrived at this conclusion after having made a study of old people in the Department of Medicine at the University of Florence. We considered three groups of patients, who were from 60 to 90 years old: a group of "pseudonormal" individuals, a group of arteriosclerotic patients, and a group of atherosclerotic patients (i.e. patients who had suffered from a previous myocardial infarction). We measured certain blood components: electrophoretic fractions of serum proteins and lipoproteins, total serum mucopolysaccharides, C-reactive protein, cholesterolemia, plasmatic heparinoid substances. If we consider the data as a whole, we can say that there is no difference in the blood picture of the three groups. We observed an increase of alpha and gamma globulins; we found a normal beta: alpha lipoprotein relationship. We observed that there was an almost constant increase in the mucopolysaccharides in the blood as well as a decrease in heparinoid substances. Another fact that we consider to be strictly connected to mucopolysaccharide metabolism is that in more than two-thirds of all patients C-reactive protein was present. Therefore, when we consider groups of persons who are more than 60 years old, we have only persons who are not normal, and that seems to be due to the fact that with ageing we have an altered metabolism at the level of connective tissues, which is reflected by the variation of those blood components that we have examined.

Olbrich: Prof. Tunbridge has made one thing very clear and he has therefore made this discussion simpler: there is no correlation between morphological and functional changes. We know that the rate of ageing varies from individual to individual, i.e. biological and chronological age are not parallel or corresponding. We estimated the total body water, and found that the extracellular water in different age groups remains constant, whereas clinically we could detect no pathology; and we found that, according to the age group, there is a decrease of intracellular water and a variation in the fat mass. I would like to suggest as a definition of age a decrease of intracellular water, with maintenance of extracellular water and a varying fat mass. As the intracellular water decreases the cell mass diminishes correspondingly, if the assumption is true that the ratio of cell solid to water remains constant. It follows that the demand of this diminishing cell mass for blood decreases, resulting in reduction of both cardiac output and renal blood flow.

The ratios of cardiac output to renal blood flow or of intracellular water to cardiac output remain constant. If the cardiac output is measured without knowing the blood volume, circulation time and intracellular water, then these single measurements are not valid.

The measurement, for example, of renal function alone and correlating it to age alone cannot avoid the error of not including pathological conditions in a group whose only object is to investigate age changes. A diminished cardiac output can be the result, in an ageing person, of a disease leading also to a diminished blood supply to the kidney and reduced renal function. This confusion is very frequent in gerontological research, especially with a "physiological" reduction of function as correlated to age alone. From this I draw the conclusion that single measurements or even multiple measurements not carried out simultaneously are valueless.

Friedman: I find myself in agreement with Dr. Olbrich insofar as the rat data which we have support his contention for man. I am not sure how far one can go to establish primacies on this sort of thing, but certainly what he says about relating measurements to one constant measure is an extremely good point.

I would point out that that shift in body water has been mentioned in the past; Lowry and Hastings have dwelt on it fairly extensively (Lowry, O. H., and Hastings, A. B. (1952). *In* Cowdry's Problems of Ageing, 3rd edition. Ed. A. I. Lansing. Baltimore: Williams & Wilkins). The point about relationships of metabolism in general to fat-free body mass has been driven home fairly often and perhaps not enough attention has been paid to it.

Olbrich: Hastings measured extracellular water with sodium thiocyanate which did not, in fact, measure the extracellular space, as this substance penetrates the cells. The penetration takes place at different rates in different age groups, so his values for extracellular water were surprisingly high and the variation in his extracellular water measurements was considerable.

When total body water is measured with antipyrine or NAAP (*N*-acetylaminoantipyrine) and the results are plotted and extrapolated to zero time, there is also great variation. Five members of my staff independently plotted the obtained antipyrine values and extrapolated them, and their results varied greatly. Prof. McCance suggested using urea, as urea penetrates all the cells, and I find the urea method gives fairly constant measurements. We know the error and we know the extent of the error.

For measurement of the extracellular water we are using either inulin or sodium thiosulphate. It is only when the same experimental methods are applied in both humans and animals that corresponding values can be obtained and only then will data on humans and on animals correspond.

Best: Perhaps Prof. Nicolaysen would say something about how we are going to proceed in the investigation of nutrition in relation to ageing, and whether or not animal results can be applied in the

human being. Perhaps he would also review the rôle of essential fatty acids in cholesterol metabolism.

Nicolaysen: I am quite optimistic both as regards animal and human studies provided they are planned and executed. Out of human pathology comes, as we all know, much valuable inspiration for fundamental studies in animals; and *vice versa,* carefully planned work on animals—on isolated organs, tissues and single cells—is fundamental in physiology. Any number of valuable results from such animal work is, in fact, the very basis of modern medicine.

I think we shall profit by following the same pattern, i.e. work on animals and man; however, results achieved in animals must, whenever possible, be tried out for validity in the human species. Since the science of nutrition is a true child of physiology and biochemistry the above would, in my opinion, apply generally irrespective of age. Species differences can be elucidated without too much difficulty.

The Chairman's question caught me somewhat unaware, when he asked me if I would review briefly the rather exciting problem concerning the rôle of essential fatty acids in cholesterol metabolism. It might therefore serve a useful purpose to take this problem to illustrate what I mean.

Considerable sensation has been caused by the recent achievements in the field of cholesterol metabolism. In fact, it has been postulated that essential fatty acids are responsible for normal cholesterol transport and that the deficiency of these acids is responsible for cholesterol accumulation and deposition in the arterial walls. The implication has been that this new knowledge, properly applied in human dietetics, would be instrumental in preventing atherosclerosis and, in consequence, coronary thrombosis and cerebral infarction—the two diseases responsible for about one-third of all deaths in our countries. This rests on the assumption that the incidence of coronary thrombosis and of cerebral infarction is intimately correlated to the severity of the atherosclerosis. However, competent clinicians will deny this and they focus attention primarily on the thrombosis problem. On the other hand, it seems to be generally accepted that thrombosis will not develop in arteries free from cholesterol plaques. However, it may be of value to recall some analytical data regarding different fats and their effect on blood cholesterol.

An oil such as olive oil contains about 9 per cent of essential fatty acids and depresses blood cholesterol. Lard, on the other hand, increases blood cholesterol; it contains 7 per cent of essential fatty acids. It is even more puzzling that egg yolk, which increases blood cholesterol very markedly, contains 60 per cent of fat as dry matter with 18–19 per cent of essential fatty acids in the fat.

It has been established that the fatty acids in cholesterol esters are the most highly unsaturated ones in any type of lipid, and fat load as well as cholesterol load on the metabolism will hasten the development of essential fatty acid deficiency in rats. It is therefore probable that the essential fatty acids play a rôle in the transport of cholesterol. The analytical figures for essential fatty acids just quoted, however, indicate that the solution is not as simple as all that.

The effect of different types of fat on blood cholesterol must necessarily be established in studies on humans. Most of the fundamental problems, such as integration of exogenous and endogenous cholesterol in metabolism, transport and eventually, in abnormal tissue, deposition will, according to my judgment, be most profitably studied in animals, and my belief is that most of the fundamentally new results will be obtained by means of animal work.

This is, in my view, a typical example in reply to your primary question: you need studies in humans, but surely the solution to the fundamental problems will originate from animal experiments. We need the descriptive work in humans before we turn to the penetrating analytical and biochemical work in animals.

Verzár: I was glad that Prof. Tunbridge brought a little optimism into our work on humans again, because listening to all the difficulties and all the criticism during these two days one tends to lose the belief that one can work on humans at all.

We ourselves, in spite of being animal physiologists, thought that our main subject should be man. We started a team which has been working in Basle now for two years, selecting so-called normal men whom we intend to study during the next 30 years. We found a factory which has a fairly continuous working unit, the young men enter it at about 20–22 and spend all their working years there. With the help of Dr. Karl Miescher it was possible to get 50 new persons a year from this factory. The second lot is now being studied. We hope to get up to 200 individuals, each of whom should be studied every second year and the continuous ageing during their life should be worked out. This is rather an optimistic scheme and its success depends on two things: first, that such individuals shall be available and that these 200, or at least half of them, will be there in 30 years time; secondly, that our organization works in such a way that the subjects should not need to lose more than one working morning in the year, which is the maximum which the factory will allow. Also, we must make no measurements which are painful to them, e.g. arterial or venous punctures. Furthermore, if the scheme goes on, some of us who are carrying out this study now will have been replaced by others and the methods, therefore, have been standardized. The subjects come in three at a time to the Medical Polyclinic, where

clinical measurements such as weight, vital capacity, electrocardiogram, and so on are established. They go then to the Physiology Department where the pulse wave velocity is measured by a standardized method; and then they go to the ophthalmologist, who is the centre of the whole team, where their visual acuity is studied by several different exact methods.

I should like, on this occasion, to give mention and honour to the mathematician Felix Bernstein, who inspired this scheme. He started this problem in New York, where he had occasion to get the prescriptions for eye-glasses from three generations of doctors, who had continuously studied the eyesight of whole families over several decades. He began to calculate the probability of age on the basis of presbyoptical changes. We shall see whether, in this way, we can give a description of human ageing in terms of different body functions which may or may not run parallel; and furthermore, whether it is possible to predict ageing on the basis of certain changes which can be observed.

We should be glad if not only in Basle but elsewhere similar schemes were started.

* * * * *

Best: The experimental biologists of our group have presented extremely well the data which are available and have suggested plans by which more complete information may be secured. It is obvious that the medical man has many advantages in studies of the kind in which we have been interested. There is no National Health plan for the lower animals. On the other hand, comparative biology can teach us a very great deal and we are tremendously indebted to those who are vigorously pursuing knowledge in this field.

We have seen how a colony of inbred animals can make possible great contributions to our subject. We admire the scientific investigators whose skill in mathematics, physics and chemistry, as well as in biology, enable them to look well below the surface of our problems and we expect great fundamental contributions from them. We respect also the more conventional biochemists and physiologists who are attempting to illuminate our subject. Most of us admit our prior preoccupation with matters pertaining to the human species, and many investigators, in all parts of the world, feel that we are at the beginning of an era which will be marked by phenomenal discoveries in the prevention of abnormally rapid rates of ageing in the human subject. We all believe that even the so-called normal rate is far too fast and we confidently expect that biological science will continue effectively to decrease its speed. Some who work in the field of genetics feel it is a little difficult to bring their findings to

fruition but we realize the great fundamental importance of this method of approach. Others work in nutritional, neurological or psychological spheres and here we sometimes see phenomenally rapid application of the findings.

We have had a fine opportunity to air and to compare our views at this Colloquium, and we are very much indebted to the Ciba Foundation, to Dr. Wolstenholme and to the members of the staff for a fine meeting. I am sure that we have all appreciated new points of view and have gained new facts which will affect our own research programmes.

AUTHOR INDEX TO PAPERS

SUBJECT INDEX

Printed by Spottiswoode, Ballantyne & Co. Ltd., London and Colchester